YALE UNIVERSITY
MRS. HEPSA ELY SILLIMAN
MEMORIAL LECTURES

BLOOD

A STUDY IN GENERAL PHYSIOLOGY

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IN GENERAL PHYSIOLOGY

BY

LAWRENCE J. HENDERSON

Professor of Biological Chemistry in Harvard University.

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THE SILLIMAN FOUNDATION

In the year 1883 a legacy of eighty thousand dollars was left to the President and Fellows of Yale College in the city of New Haven, to be held in trust, as a gift from her children, in memory of their beloved and honored mother, Mrs. Hepsa Ely Silliman.

On this foundation Yale College was requested and directed to establish an annual course of lectures designed to illustrate the presence and providence, the wisdom and goodness of God, as manifested in the natural and moral world. These were to be designated as the Mrs. Hepsa Ely Silliman Memorial Lectures. It was the belief of the testator that any orderly presentation of the facts of nature or history contributed to the end of this foundation more effectively than any attempt to emphasize the elements of doctrine or of creed; and he therefore provided that lectures on dogmatic or polemical theology should be excluded from the scope of this foundation, and that the subjects should be selected rather from the domains of natural science and history, giving special prominence to astronomy, chemistry, geology, and anatomy.

It was further directed that each annual course should be made the basis of a volume to form part of a series constituting a memorial to Mrs. Silliman. The memorial fund came into the possession of the Corporation of Yale University in the year 1901; and the present work constitutes the twenty-first volume published on this foundation.

PREFACE

IN this book my Silliman Lectures are presented in an expanded form and with the addition of such material as seemed necessary to make the work methodical and useful. In accordance with a suggestion of the Committee on the Silliman Lectures I have set forth the results of my own work, especially those of the past decade, and have not endeavored to review the subject as a whole. This method of treatment is justified by the fact that the Silliman Lectures of J. S. Haldane and A. Krogh and the works of Barcroft, E. J. Warburg, and others have done what I could not do so well.

The experiments upon which the conclusions of this book rest have been in some instances exceptionally difficult and intricate. Without the aid of my collaborators they could not have been performed. Many of the most important results are due exclusively to the great skill of A. V. Bock and D. B. Dill. The concerted effort, more useful than routine coöperation, in D. D. Van Slyke's laboratory and my own has also been indispensable to success. I am indebted to Professor Dill for the valuable Appendix and for much kind assistance in the preparation of the book.

L. J. H.

Cambridge, Massachusetts
April 3, 1928.

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CHAPTER I

GENERAL PHYSIOLOGY

THE subject of this book is the red blood of vertebrates. We shall study this substance as a physicochemical system and as a tissue, seeking in its properties the exemplification of some of the general characteristics of protoplasm. In its physiological function and interrelation with other parts of the body we shall look for an illustration of organic integration and adaptation. We shall also study it comparatively from species to species, in rest and activity, in health and disease. So far as possible these studies will be quantitative and mathematical.

Before approaching such questions and the study of the facts, certain preliminary reflections will be profitable. We shall accordingly consider, not only what we are to do, but how we are to conceive it and to do it. To this end we must scrutinize the present state of the science of general physiology, an imperfect and still most incomplete fabric bearing slight resemblance to the richness of living reality and hardly more to the perfection of some other sciences.

Like all our enterprises the sciences are changeable. They grow and develop. At first descriptive and classificatory, then rational, in this twentieth century some will say that they are destined to become metaphysical. But before they can attain to this last condition it seems probable that they must pass through a stage in which all is clarity, simplicity, and order, where there is no room for philosophic doubt and where, by a singular paradox, the adoption of approximations and philosophically dubious abstractions yields certainty, or at least the closest approach to certainty that man has ever known.

It is in proportion to our success or failure in conceiving facts simply that sciences are abstract or concrete, rational or descriptive. In these respects the contrast is great between the physical and the biological sciences. The figure of the earth, its path about the sun, and its relations to the other planets are readily conceivable in a first approximation as simple; but the forms of life seem complex, their activities manifold, and their concatenations interminable. Therefore, unlike celestial mechanics, the science of biology, which is the record of efforts accurately to describe and clearly to understand living things, is chiefly a science descriptive of concrete fact. It bears little resemblance to the more perfect science and as yet is in no danger of a relativist revolution. It has never attained, perhaps, as some have argued, it can never in any respect achieve and should not strive for the abstractness, the elegance, and the simplicity which are the mark of the classical epoch of many of the physical sciences and the ideal of those who follow Newton and Willard Gibbs.

Throughout the vast extent of biology, there may be found but few valid and useful abstractions, such as species, evolution, organism, heredity, protoplasm, metabolism, and the like. Even these fall short in definition, and for that reason in usefulness, of successful physical abstractions like velocity, inertia, temperature, component, and phase. The biological abstractions are still imperfectly represented by vague words; long since the physical abstractions have taken the form of precise terms, and uncertainties have thus been replaced by problems concerning the choice of definitions, of postulates, or of hypotheses. Biology is also rarely quantitative, still more rarely both quantitative and rational. In short, a generalized scientific description of organic phenomena is, for the most part, as yet unattained and probably still unattainable. Meanwhile, a well-ordered science of theoretical biology remains impossible.

Such facts point not to defects but to incompleteness in

a difficult enterprise which is even now immature, and which may never conform closely to the pattern of the rational physical sciences. There can be no doubt, however, that we all feel the need of clear and abstract thinking about the phenomena of life, and that we must seek all the help that can be got from methods which have made our thinking about mechanical and chemical phenomena clear and abstract.

To be sure, the world has long been acquainted with fragments of a science of general biology, for Aristotle was a general biologist. His marvelous powers of abstraction and his only less marvelous powers of collection, observation, and classification gave rise to a treatise on biology[1] which, with all its faults, is general, and which held the field for many centuries. The intrinsic activities of living matter were, however, completely unknown to him, and so remained until the times of Mayow[2] and of Lavoisier and Laplace.[3] Hence, general physiology, which, when broadly defined, makes up a great part of general biology and affords almost the only opportunity for the biological use of many of the most powerful scientific methods, is a modern science. It was first clearly conceived by Claude Bernard who, when he died fifty years ago, left behind a program for the new science that he himself had gone far to carry out.[4] Claude Bernard held and clearly explained that it is the task of general physiology to study the phenomena of life which are common to animals and plants. These phenomena are physical and chemical phenomena; they are to be investigated by physical and chemical methods; they are conditioned by the same physical and chemical forces that may be dis-

[1] Aristotle, *Historia Animalium, De Partibus Animalium, De Motu Animalium, De Incessu Animalium, De Generatione Animalium.*

[2] J. Mayow (Alembic Club Reprints, No. 17), *Tractatus Quinque Medico-physici,* Oxford, 1673.

[3] Lavoisier and Laplace, Paris, *Académie des Sciences,* 379 (1780).

[4] C. Bernard, *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux,* 2 vols., Paris, 1878, 1879.

covered among inorganic phenomena. But in the living being they are also always harmoniously organized and integrated, and this is their most striking characteristic. It gives rise to the central problem of general physiology. Especially with the help of his theory of the *milieu intérieur,* Claude Bernard was able to treat many facts, including his own discoveries, from this point of view and thus to embody in durable form ideas which were in advance both of the spirit of his contemporaries and of his own physico-chemical knowledge.

At the same time Gibbs's memoir "On the Equilibrium of Heterogeneous Substances" was in course of publication in the *Proceedings* of the Connecticut Academy.[5] This mathematical research, the greatest effort of sustained abstract thinking in the history of America, was destined to awaken chemists from their empirical slumbers and to make possible the study of the problems of general physiology at their deepest level, as Claude Bernard had conceived them, to provide a foundation, in short, on which a rational science of general physiology could be reared. But the twentieth century had begun before physiologists were ready to make use of the theories of Bernard and Gibbs. Even today, in spite of many scattered investigations and of the influence of the important treatise of Bayliss,[6] the science is still in its infancy. Its significance is now widely recognized, but Claude Bernard's program has already grown old and has not been revised. A fresh survey of the field is needed.

For Claude Bernard, "General Physiology is the fundamental biological science toward which all the others converge. Its problem consists in determining the elementary condition of the phenomena of life."[7] To this statement there is probably less objection today than

[5] J. Willard Gibbs, *Scientific Papers,* I, 55, London, 1906.

[6] W. M. Bayliss, *Principles of General Physiology,* 1st ed., London, 1915.

[7] C. Bernard, *Introduction to the Study of Experimental Medicine* (tr. by H. C. Greene), p. 65, New York, 1926.

there was a half century ago. But what is the elementary condition of the phenomena of life, and does it admit of clear formulation?

The study of this problem may conveniently begin with an examination of the question: How far and in what respects may the activities of all living things be regarded as identical? To this question, the biologists' use of the term protoplasm suggests an answer.

It is customary to say that all cells contain protoplasm, and to imply that the word is well chosen in that this substance is the very stuff of life, the undifferentiated material out of which all organic matter is formed. Nevertheless, a sufficiently clear and intelligible definition of the term is never given, because such a definition is at present impossible; it is likely long to remain so, but not indefinitely. A first difficulty in approaching this question may be noted in the well-founded theory that each type of cell in each species of organism contains as constituents of its protoplasm one or more proteins which are peculiar to it alone. This probably implies the presence of specific enzymes and of a specific physico-chemical structure. Surely, therefore, the term protoplasm is a high but vague abstraction. Yet, in default of a better, it must be made to serve.

In truth, it is not the inaccurate, that is, the roughly approximate, character of the definition of protoplasm which is at fault. This is rather an advantage—necessity made a virtue in a manner common to all the sciences—which renders the term more widely serviceable. The difficulty is that the term is vague. But from a physico-chemical point of view the word protoplasm is not quite meaningless, for it has already received certain increments of a definition. We know, for example, that protoplasm always contains water. Other constituents are bicarbonates, chlorides and phosphates of sodium, potassium, and calcium. Free carbonic acid also never fails and probably certain other inorganic substances. It is, further, by no means impossible that a number of simple

carbon compounds like glucose might be added to the list. Therefore we may begin with the following statement: Protoplasm is a physico-chemical system among the components of which are water and carbon dioxide, hydrochloric and phosphoric acids, the hydroxides of sodium, potassium, calcium, etc., and other substances. The concentration of each of these components is subject to wide variation.

This statement, though unexceptionable, is not very useful. Even if it were possible to add to the enumeration all the other unfailing components, we should still be far from a good working definition. In particular it must be pointed out that there would not yet be at our disposal any substance except water to which the most conspicuous properties of protoplasm are attributable. But the statement is not useless and it is precise. Indeed, the study of such systems, to which the term buffers is commonly applied, has considerably extended physiological knowledge. It was such investigations which first made possible the quantitative description of a physico-chemical equilibrium in protoplasm, and the experiments which are to be reported in these lectures have grown out of and rest upon these earlier studies.[8] Finally, we may note that it is impossible to go beyond such a restricted specification of the components of protoplasm, if we are to be quite exact.

These facts illustrate an important aspect of general physiology and of other sciences, for, in their chemical aspects, they are of no great interest. But that which may be of only special and even trivial importance when studied from the standpoint of a relatively abstract science like physical chemistry is sometimes general and important when studied from the standpoint of a less abstract science like physiology. It is, perhaps, hardly possible that the physical chemist should ever have taken much interest in a system whose components are so numerous

[8] L. J. Henderson, *American Journal of Physiology,* XV, 257 (1906); XXI, 427 (1908).

as those before us, but the study of this system is, for the physiologist, the beginning of an ideal reconstruction of protoplasm, and therefore an approach toward a description of the elementary condition of the phenomena of life.

In order to proceed with such a reconstruction, it is next necessary to take account of classes of substances which are invariably represented in protoplasm. Such are proteins, carbohydrates, lipoids, enzymes, if it be permissible here to refer to what is probably, from the chemical standpoint, no true class, and perhaps various other substances. In this manner it is possible to go far in designating the components, in Gibbs's sense, of protoplasm, and we can already see that this substance is not conceivable as a single physico-chemical system, but rather as a pretty sharply defined class of systems. These systems always contain certain components and also variable representatives of certain classes of components.

Experimental research on artificial systems of many components is difficult, and as yet has hardly progressed beyond the addition of a single protein to the buffer systems mentioned above. Meanwhile the physico-chemical study of protoplasm itself is, for many reasons, not far advanced. Yet we already know that one of the important tasks of general physiology consists in the descriptive physico-chemical study of systems prepared from various pure constituents of protoplasm of widely different origin, so as to discover phenomena which are not easy to observe in anything so complex as protoplasm itself. In performing this task, it is not surprising that the physiologist should find it necessary to do his own work, for physical chemistry has developed through the study of simpler systems. Years ago a similar situation arose in the study of steel, and the problem was solved by specialists. For researches of this kind, Gibbs's memoir is an indispensable guide.

It is safe to say that a broad application of descriptive physical chemistry to general physiology will yield important results. For, in addition to the multitude of com-

ponents which everywhere complicate the physiological problem, there are further complications due to the intricate colloidal structure of protoplasm which also invite physico-chemical experimentation. Leibnitz once remarked that, "the machines of nature, that is to say, living bodies, are still machines in their smallest parts *ad infinitum.*"[9] Today we may perhaps cautiously apply this idea to the relations between the molecules and ions of protoplasm. It seems probable that a large proportion of these are arranged according to some plan, not quite so rigidly as in a crystal to be sure, but by no means at random. In short, organic structure is probably nowhere lacking in protoplasm. Only with the help of physical chemistry can we hope to overcome the difficulties which thus arise.

The elementary physiological activities in diverse species of living things can never be identical, and can be so conceived only in a very rough approximation. So far as they concern only water and inorganic acids, bases, and salts, they may be nearly alike. But when proteins and other substances are involved, to say nothing of varying physico-chemical structures and metabolic activities, they must be more or less different and specific. Yet the ultimate physico-chemical phenomena of protoplasm are to be regarded as the activities, in each case, of a particular member of a well-marked but little-known class of physico-chemical systems, and protoplasm may be defined as this class of systems. Such is the ground for regarding certain types of physico-chemical phenomena as the "elementary condition of the phenomena of life," and for assimilating a special field of descriptive physical chemistry to general physiology, as one of the foundations of the science.

This exposition of the physico-chemical constitution of protoplasm must seem empty indeed to the biologist; and so it is. But he may be reminded of the old axiom of biology that the sum of the parts is always less than the

[9] Leibnitz, *Monadology,* §64.

whole, and again that half a loaf is better than no bread. More important is the reflection that it may be well, in any science, to begin at the beginning. But what makes such a description important is that the study of this small number of variables is sufficient to explain at least a few important facts, and to raise hopes of the explanation of others. Also it is one of the best rules of scientific method to operate with the smallest possible number of variables, to carry the analysis of their mutual dependence as far as possible, and to add others only in so far as it may be necessary in order to increase the accuracy of the results or to enlarge the field of investigation. Without the help of some other variables it may seem hopeless to take account of such facts as irritability and reproduction, but this is uncertain.

It must not be overlooked, however, that we have thus far made little reference to the more highly integrated properties of protoplasm. Therefore, although it may not now be possible to explain these in physico-chemical terms, yet, for many reasons, it is necessary to examine some of the general properties of the class of physico-chemical systems to which protoplasm belongs. Here there is little enough that can be said, for physical chemistry has developed largely as a theoretical science, and the field of descriptive physical chemistry—the vastest in the whole domain of physical science—has been little explored.

The large number of components of protoplasm is a condition of the first importance in determining the nature of the system. In physical chemistry and the other physical sciences it is customary, as above suggested, to deal with but a small number of variables. Often, no doubt, it is possible *ideally* to take account of large numbers of variables, and Gibbs did, in fact, consider the case in which the number of components of a system is unrestricted. But concrete instances of systems in which the number of variables is large are habitually avoided. Also, the mathematical treatment of a large number of vari-

ables usually presents very serious difficulties, as the history of the dynamical problem of three bodies implies. There is, of course, an escape from this situation when the number of variables becomes large enough to make the statistical treatment fruitful, but such methods are at present inapplicable to protoplasm, and do not ordinarily lead to rational simplification.

For these reasons the large number of components of protoplasm is a condition which seriously weakens our methods of attack upon the problems of general physiology. But also, and this is more to the point of the present discussion, it increases the complexity of these problems. For, in a system of n variables, each variable will in general be a function of the other $n - 1$. Thus a change impressed upon one variable will directly involve changes in all the other $n - 1$ variables. But then the change in each of these will involve secondary changes in all the others, and so on indefinitely. Hence, the number of secondary changes increases very rapidly as n increases. When, as in protoplasm, n is great, the complexity of the analysis becomes very great.

The nature of the case will be more readily appreciated by reference to another branch of biology in which our intuitions are better practiced. Consider a human society of n individuals and let n equal successively 2, 3, 10, and 20. When $n = 2$ or 3, a short story will sometimes suffice as a description of the society. When $n = 10$, it is doubtful if even the greatest poets or novelists have ever successfully described a single instance. When $n = 20$, a description is possible only if most of the individuals become mere puppets.

In the description of artificial physico-chemical systems it is often possible to neglect the effect of one variable upon some or all of the others. Thus, for example, with water, glutamic acid, sodium hydroxide, carbon dioxide, and oxygen as components in a system consisting of a liquid and a gas phase, the presence of oxygen may be neglected without sensible error. In physiological sys-

tems, however, there is a marked tendency for a smaller number of the influences of one variable on another to vanish in a first approximation. Thus in blood oxygen affects both hemoglobin and the acid-base equilibrium. Perhaps it may be said that protoplasm is characterized by a quantitatively large dependence of each variable on all the others, or on an improbably large proportion of the others and in an improbably large number of different modes. Certain it is that one of the most important peculiarities of protoplasm is the high degree of connection (or low degree of independence) between its components. But it should be noted that this is true of every well-integrated whole, for example, an atom, a molecule, or a watch. In protoplasm this principle of the high degree of connection between the parts is illustrated both by phenomena at the level of simple chemical equilibrium, like the instance of the interaction of oxygen and carbon dioxide through hemoglobin above cited, and also by the structural organization as revealed in studies of the mechanism of oxidization,[10] of enzyme complexes,[11] and the like. Another illustration of the same principle is to be found in the antagonistic action of salts. Here it is observed that differences between similar simple substances like sodium and potassium ions, which are often negligible in artificial systems, may be very important in biological systems. The wide range of phenomena above cited does but emphasize the importance and the generality of the principle, which describes one of the striking aspects of all organisms.

In systems where the number of variables is large and the degree of their mutual dependence high, the application of mathematics, though difficult, is of peculiar interest. In fact, as mathematical economists like Walras,[12a]

[10] Warburg and Meyerhof, *Pflüger's Archiv,* CXLVIII, 295 (1912).

[11] R. Willstätter, *Berichte,* LV, iii, 3601 (1922).

[12a] L. Walras, *Éléments d'économie politique pure,* 1st ed., Lausanne, 1874.

Fisher,[12b] and Pareto[13] have seen, it is even more important in such circumstances than in the simple phenomena studied by physical science; more than this, for many purposes it is simply indispensable, because there is no other way even to begin to think, however approximately, about the quantitative relations between a large number of variables. Under these conditions the nomographic method, particularly in the form developed by d'Ocagne,[14] has proved itself an indispensable aid to the physiologist. In this way it is often possible to construct a graphical representation of the facts and thus attain to a roughly comprehensive representation of the system as a whole. Such a construction also serves to illustrate many of the general characteristics of the physiological systems which are above discussed. When even an incomplete rational interpretation of the relations is also possible we begin to approach the methods of the physicist, for we can operate nomographically and think theoretically.

The activity of protoplasm is not less various and heterogeneous than its constitution. The modes of this activity may be classified in three categories as physicochemical, chemical, and mechanical, though such a classification, a very rough approximation, can hardly be exhaustive. A basis for the discrimination between physicochemical and chemical processes is to be found in the fact that although, next to water, proteins are the principal constituents of protoplasm, carbohydrates are often the substances which, as in muscle and in yeast, chiefly undergo metabolism. Thus there may be distinguished a relatively permanent, though active and changing system, which is regarded as the instrument that produces the more rapid metabolic or chemical process. A more objective view discriminates between the true equilibria in protoplasm, such as the reactions of ions, and other re-

[12b] I. Fisher, *Transaction of the Connecticut Academy,* IX, 3 (1892).

[13] V. Pareto, *Manuel d'économie politique,* p. 612, Paris, 1909; *Manuale di economia politica,* Milano, 1906.

[14] M. d'Ocagne, *Traité de nomographie,* 2d ed., Paris, 1921.

actions which can never be in equilibrium, though they may be in a stationary state, such as some of the steps in the metabolism of carbohydrate. Many reactions, however, fall in neither of these categories, for they must be, in some respects, in a condition almost indistinguishable from true equilibrium, while involving substances otherwise engaged in slow irreversible changes, the effects of which are more or less balanced by accessions from without. However this may be, it is at least plain that the one class of phenomena are to be studied with physico-chemical methods, the other with chemical methods, and, since there is probably no sharp demarcation between the two classes, this is the most useful criterion for a classification. It is the one which has been widely, though perhaps unconsciously, followed.

Meanwhile it should be noted that we have encountered one more characteristic of protoplasm: As a physico-chemical system it is never in equilibrium, but only, at best, in a stationary state like the candle flame or whirlpool. This is, however, not a fact of the first importance in many investigations, for in some respects, as an approximation, it is both convenient and sufficient to assume the existence of a state of equilibrium, which does indeed exist in some of the intrinsic processes. The ideas here stated are merely a more precise formulation of a conception of the organism which is derived from Cuvier[15] and perhaps even from Lucretius.[16] Possibly comparison of protoplasm with a more complex natural object such as a swamp may aid the imagination, for in this case conditions are to be found that may be regarded as equilibria, while others resemble the flame and the whirlpool. The solid parts of the swamp which are by no means inactive and changeless, though relatively so, may stand for the physico-chemical structure and activity of protoplasm; the water which flows through the swamp in quantities varying with the season and the rainfall, but

[15] G. Cuvier, *Le Règne animal,* I, 13, Paris, 1817.

[16] Lucretius, *De Natura Rerum,* bk. II.

in a regulated or at least regularized manner, may perhaps be thought of as analogous to the chemical or metabolic processes. This comparison will suggest to what extent such a discrimination as the chemical-physico-chemical one is natural and how far arbitrary.

Above the level of those just considered, the most characteristic activities of protoplasm are movements. They must always arise from the energy liberated in the chemical processes, acting through the physico-chemical processes, upon well-adapted physico-chemical structures. The analogy of the steam engine, boiler, and furnace considered as one system is familiar. Here the heat of combustion, acting through the physico-chemical processes of steam formation, expansion, etc., upon the mechanism designed for the purpose, produces motion.

Since the days of natural theology the argument from analogy has justly fallen into discredit. And it must not be supposed that the present analogies can serve any purpose but illustration. I believe, however, that argument is unnecessary to establish the proposition that the activities of protoplasm are not only various and heterogeneous, but also harmonious and mutually adapted. As for the classification into physico-chemical, chemical, and mechanical activities, this, like all classification, is arbitrary and, like most, only roughly approximate. However it may be with these difficult methodological questions, there can be no doubt that one of the important tasks of general physiology, which differentiates it from the physical sciences, is that it has to take account of simultaneous activities of widely different kinds harmoniously interacting.

Such interactions become more conspicuous as structures become more differentiated and finally take the extreme forms of hormonic and nervous integrative action. But it is evident that in the organism every activity is more or less integrative. Or, more precisely stated, every physiological phenomenon must be studied, not only in isolation, but also in relation to other phenomena with

which it will in general be found to be related in a manner usually called adaptive. This is no less true of the ionic reactions which are common to all protoplasm, of the metabolic oxidation of glucose, and of the interaction in blood of oxygen and carbonic acid through hemoglobin, than of locomotion in amoeba or of the activities in the central nervous system which have been studied by Sherrington.[17]

There is a disposition in certain quarters, among those who still permit themselves to deduce rules of scientific method from arbitrarily assumed metaphysical principles, to object to the concept of adaptation as teleological and non-mechanistic. I hold, on the contrary, that the only objection that can fairly be offered is to the vagueness of the term. It must be remembered when we consider this question that the typical physical sciences are abstract, and for that reason they often exclude *a priori* the study of facts which lead to considerations of the kind that adaptation involves. But even in pure mechanics the principle of least action and the concept of stability have taken an important place, while in engineering the terms efficiency, regulation, and adaptation are as familiar as in physiology. In these cases, concepts which some have regarded as teleological and non-mechanistic have tended to take the form of mathematical functions which are implicit in the most mechanistic of all our scientific formulations, and this is also sometimes true in the field of physiology. Thus the stability of the alkalinity of blood and protoplasm may be measured by the value of a mathematical function which is implicit in the general mathematical description of the equilibrium between acids and bases, and the regulation of alkalinity, that is to say, its relative constancy over a long period of time and under widely different circumstances, may be quantitatively described if we extend our studies from the physicochemical equilibria of blood and protoplasm to the inter-

[17] C. S. Sherrington, Silliman Lectures, *The Integrative Action of the Nervous System,* New York, 1906.

action of these with the activities of the lung and the kidney, and with the varying processes of metabolism.

It is unreasonable to expect a precise definition of the term adaptation or the substitution of clearly defined terms for it, until greater progress has been made in mathematical physiology. Meanwhile there is one further remark which may be made. No characteristic of organisms is more certain than survival. Living things do in fact persist over long periods of time as physico-chemical systems which remain approximately in a stationary state. Now if we reject the considerations which are involved, however vaguely, in the use of the term adaptation and are excluded if we exclude it, if, in short, we limit ourselves to those considerations which belong to conventional abstract physics and chemistry, the survival of a single organism may be said to be almost infinitely improbable, and the continued existence of the flora and fauna of the earth an unaccountable miracle.

Therefore, since survival is one of the best established of all facts, there is nothing to do but to accept it with its implications. It must be admitted that the precise definition of these implications is difficult, but continued study will make it less so. For example, it is well known that in organisms adaptations are not only incomparably more numerous than in machines, but that the organic adaptations often are of a kind which has hardly yet been introduced into machinery. Not to mention regeneration and repair (which, though unavoidable descriptive terms for the biologist, certainly recognize aspects of phenomena which are no less teleological than other forms of adaptation), there is the fact that in the organism, when activity changes, structure changes with it. In cases where this process admits of quantitative study it is sometimes possible to prove that an increase in the efficiency of the mechanism has been achieved. The study of pathology yields an analogous result. Here the body, working under difficulties resulting from the destruction or injury of

parts, changes throughout in a manner which can often be shown to preserve efficiency.

Far from seeking to avoid or to minimize the adaptive character of organic phenomena, we should, I believe, invariably take this for granted. The law of adaptation in organisms, founded upon the fact of survival, seems to be quite as well established as the second law of thermodynamics, and almost equally serviceable. Caution is, however, necessary, and *Candide* should never be forgotten, for one must carefully avoid, in studying the microcosm, the ancient fallacy of the best of all possible worlds. Adaptation is relative, it involves a question, not of what is best, but of what is efficient under certain conditions, of what promotes survival in a particular environment, or of the disabilities resulting from a given lesion.

Elsewhere I have discussed this question at length and shown that organism rather than mechanism is the concept which has long guided many of the biological sciences.[18] Recently Whitehead in a survey of the philosophy of science from the standpoint of a theoretical physicist, has rejected classical mechanism altogether as a philosophy even of the physical sciences.[19] This he aims to replace by a theory of organic mechanism. Whitehead's criticism of the older view seems to provide more than sufficient ground for the cautious restriction of the scope of the mechanistic principle which is here proposed. It is, however, my purpose to go no farther than necessary in this direction, since we are here concerned not with philosophical questions, but with a description of scientific method and a characterization of general physiology.

We have already seen that in protoplasm the number of variables is large and the number of quantitatively significant relations between them improbably large. We may now add that an adaptive character is in general to

[18] L. J. Henderson, *The Order of Nature,* chap. V, Cambridge, Mass., 1917.

[19] A. N. Whitehead, *Science and the Modern World,* New York, 1925.

be ascribed to these relations. It is as yet impossible to say precisely what this implies, but none the less evident that such terms as efficiency, stability, and the like will arise from the mathematical formulation of our observations and experimental data, just as they do in the study of the problems of engineering. What is needed is mathematical analysis of the facts, unincumbered by the prejudices of either vitalists or mechanists.

In the preceding discussion we have passed back and forth from the consideration of protoplasm to that of cells, tissues, and whole organisms. This is permissible, if due caution be exercised, because in physiological studies we are concerned with imperfectly isolated systems. In imagination we may take as the physico-chemical system, in Gibbs's sense, either a bit of protoplasm, or a larger portion of an organism, or the whole organism together with its environment, which may be, for example, a respiration calorimeter. Only in the last case will the condition of independence of the system be approximately fulfilled. A second justification for such a loose method of discussion is to be found in the fact that we are here concerned with general physiology and that many of the general characteristics of the phenomena of life manifest themselves at all levels of physiological activity. It is, indeed, obvious that in a complex organism with highly differentiated tissues and organs the physiological processes are more complex than in an amoeba. New types of structures and processes, larger in scale, are superimposed upon those which occur within the cell. But nobody is in danger of forgetting this, while even most biologists still fail to realize the complexity of invisible structure and the almost inconceivably varied activities of what they are pleased to call simple protoplasm. So far as experimentation is concerned it may be said, paradoxically, that a single cell is as complicated as any organism, since it is more complicated than the most complicated system which is as yet within the scope of our methods of investigation. On the whole what is true of the small-scale

physiological systems remains true of the large-scale systems, because each of the latter is made up of a multitude of the former, while the general characteristics of the large-scale processes in many respects conform to those of the small-scale processes.

We have seen that protoplasm is clearly conceivable, not as a single system, but only as a class of physicochemical systems, because some of its components, notably proteins, vary from species to species and from tissue to tissue. Moreover, in spite of its remarkable stability, the state of the system varies with its activity; and so does its composition. It also varies pathologically. Therefore, since there is no such thing as protoplasm in general, and no such thing as a permanently stationary state for any one specimen of protoplasm, a general conception of this class of systems and, therefore, a science of general physiology must rest, in part, upon comparative studies. For the purposes of general physiology it is certainly not necessary to carry such researches as far as may be necessary for special purposes, but the extent and the nature of the differences between different species and of the fluctuations about a mean or basal state, or about some curve representing the changes accompanying change of age, must be known. Since the work of Galton has become common knowledge it should not be necessary to labor the point that a class of objects may be quantitatively described as a class, or to suggest that it may be interesting to extend certain investigations to the important activities of living things.

Among comparative studies those which have to do with pathological states possess a special interest and, I think, a peculiar importance, for the modifications of the organism produced by disease are sometimes far more extensive than anything within the reach of our crude experimental methods. Experiment is often best as a means of settling a clear issue, and, needless to say, surgical and pharmacological modification of organisms is the very foundation of experimental physiology, but nothing

among natural phenomena is more wonderful or more instructive than the condition of an individual who has long been suffering from a progressive disease like chronic nephritis, who is approaching his end, but who still remains adapted in every part and in every activity to the changed and almost impossible conditions of life. Under these circumstances the harmonious unity of the organism is perhaps even more apparent than in health, and it is certainly much easier to estimate the relative physiological importance of different things. It is strange and regrettable that during the past half century of rapid progress in medical science, theoretical pathology should have been so neglected.

Protoplasm is a system of exquisite sensitiveness. In order that it may survive it must be protected from too great, or too rapid, or too irregular fluctuations in the physical, physico-chemical, and chemical conditions of the environment. Stability may sometimes be afforded by the natural environment, as in sea water. In other cases an integument may sufficiently temper the external changes. But by far the most interesting protection is afforded, as in man and higher animals, by the circulating liquids of the organism, the blood plasma and lymph, or, as Claude Bernard called them, the *milieu intérieur*. In his opinion, which I see no reason to dispute, the existence and the constancy of the physico-chemical properties of these fluids is a necessary condition for the evolution of free and independent life.[20] This theory of the constancy of the *milieu intérieur* was an induction from relatively few facts, but the discoveries of the last fifty years and the introduction of physico-chemical methods into physiology have proved that it is well founded. There can be no doubt that the cells of warm-blooded animals are bathed by liquids of quite exceptional stability of composition and of physico-chemical properties, so that their protoplasm is in general not obliged to protect itself, if one may use

[20] C. Bernard, *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux,* I, 113, Paris, 1878.

such an expression. Needless to say, protoplasm is also otherwise protected. Thus it is often covered with a cell membrane which is not permeable to all substances and may manifest selective permeability even toward similar molecules. Through the study of these facts the relations of protoplasm to its immediate environment are gradually becoming intelligible.

It would not be difficult to continue at length this discussion of general physiology. For example, we have hardly touched on metabolism, or on reproduction, including the reproduction or synthesis of specific proteins and enzymes, while the problems of heredity have not been even mentioned, nor have those of behavior, of constitution, type, and individuality. Such questions are of the greatest interest to all physiologists. If it were possible to relate them to our present knowledge of the physicochemical properties of blood and protoplasm, we should have to take them into account. The time has, however, not yet come when the facts can be usefully brought together, and at this stage in the development of the science speculation is idle. Only in the case of metabolism shall we find it possible to go a little farther.

This abstract description of the methods by which we may at present approach the study of the "elementary condition of the phenomena of life" will, I hope, prove sufficient for the present purposes. As a philosophy of the organism it is manifestly defective. But in biology too much abstraction is a dangerous thing and it is time that we approached the facts.

CHAPTER II

COMPONENTS AND FUNCTIONS

THE blood of vertebrates is a physico-chemical system of great complexity. For this reason knowledge of its composition is incomplete and methods of studying and describing it are sometimes only roughly approximate. As a tissue or part of the body it performs many functions. Therefore an investigation of any problem of the physiological activity of blood must needs be fragmentary and, like all such researches, is always pursued in disregard of phenomena which later have to be taken into account.

Nevertheless, relatively to other parts of the body blood is a simple system, and those of its physiological functions which involve activities of large magnitude are few. They seem to be exclusively physico-chemical, for metabolic activity and irreversible chemical processes of all kinds are nearly inappreciable, and movement, except in the case of the leucocytes, is imparted from without. In the ordinary course of the circulation there are no enzymatic processes of importance. The red cells, often without a nucleus, are relatively structureless, the plasma entirely so. Thus if not in the biological sense, at least in the mathematical sense, blood is a degenerate case of a tissue, constituted of degenerate types of protoplasm. But this is as much as to say that blood is in some respects peculiarly well suited to quantitative experimental study.

There is, in fact, no more important condition in scientific research than the isolation of the phenomenon to be studied from disturbances of other kinds. For instance, the absence of all but dynamical phenomena from the subject of his investigation made Newton's *Principia* possi-

ble. Under other conditions it is in the limitation of such disturbances that the master of experimentation reveals himself. But in physiological research such limitation is always difficult and often impossible. Thus the case of blood is for the physiologist not altogether unlike that of the solar system for the student of dynamics. No doubt it would go too far to compare favorably the advantages which absence from blood of metabolic activity, of enzymatic activity and the like confer on the physiologist with the far more perfect convenience which the student of celestial mechanics enjoys, but it would distort the facts to overlook these advantages.

The question is sometimes debated whether blood should be honored with the rank of tissue, or whether the red cells contain protoplasm. Perhaps no one has ever suggested that blood plasma should be classified with protoplasm. Yet putting aside verbal questions as meaningless or unimportant for our purpose, and remembering that in a continuum classifications are arbitrary and boundaries conventional, it is easy to show that blood corpuscles and blood plasma alike resemble protoplasm. Also, as we shall see, this resemblance is sufficiently great so that blood, red cells, or plasma may be regarded as a physico-chemical system belonging to the same class as protoplasm. Accordingly it is possible to obtain much useful information regarding protoplasm from the study of blood, and this information is of a general character because of the very differences between blood, cells, and plasma on the one hand and more typical protoplasm on the other. Is it not, in fact, axiomatic, or possibly an identical proposition or a definition, that those properties which are common to widely different members of a class are the general properties?

We must now describe blood as a physico-chemical system. This description will be in the beginning not the most exhaustive but the least exhaustive, not the most accurate but the least accurate approximation to the facts of which we can make use.

In a first approximation blood (or more precisely blood in which the red cells have no nucleus) may be regarded as a physico-chemical system consisting of two phases: red cells and plasma. The relative volumes of the two phases are very variable from species to species, and, within one species, in different physiological states and in different pathological states. In extreme instances the cells may make up more than 60 per cent or less than five per cent of the volume of the blood. In man they may perhaps vary almost throughout these limits, but under normal conditions they constitute about 40 ± 5 per cent of the volume of the blood. The cells vary in size and form from species to species and, though much less, under pathological conditions. The form is invariably such that the area of surface is much greater than that of a sphere of equal volume, while the distance of any point in the interior from the surface is small. In man the volume of a single cell is slightly less than 100 cubic microns, the surface area slightly greater than 100 square microns, and, per liter of blood, the surface area of red cells is about 500 square meters. Every point in the interior of such a cell is less than two microns distant from the surface. Accordingly a liter of human blood may be roughly compared with a two-phase system in which each phase exists as a layer one micron thick and 500 square meters in area.

The principal components of the system are water, certain bases, hydrochloric, carbonic, and other acids, proteins, and oxygen. Evidently, in so far as it is possible to study the system without going beyond these components, all conclusions except those dependent upon the specific properties of the blood proteins must be applicable to protoplasm in general. This is often the case.

The masses of the components present in blood are variable. In man water makes up nine-tenths of the mass of the plasma, two-thirds of the mass of the cells, and accordingly approximately four-fifths of the mass of the whole blood. Nearly one-third of the mass of the cells is

hemoglobin. The proteins of blood plasma constitute about seven or eight per cent of the whole mass of their phase. The other components above mentioned account as a whole for less than two per cent of the mass of the blood, and, disregarding leucocytes, plasma fats, and lipoids, and such other substances as may be excluded by the approximation involved in the assumption of a two-phase system, the multitude of other components, neglected in this approximate statement, appear to make up but a very small fraction of the total mass.

It is important also to take note of the magnitudes of the concentrations of the more important constituents of blood, expressed in the familiar chemical manner as moles and millimoles per liter. In these units water is present in a concentration of about 45 moles per liter, but hemoglobin, even on the assumption of an improbably low molecular weight, has a concentration of only eight or nine millimoles per liter in normal human blood, and the concentration of the plasma proteins is much less than this. In an approximate description many of the colloidal properties of the proteins may be neglected, but the high ratio of mass to concentration and the consequent high viscosity with low osmotic pressure are important, while the mere size of the molecule of the protein is a significant factor in all osmotic exchanges within the organism. The total concentration of base amounts to about 150 millimoles per liter, of chloride to about one-half this, and of bicarbonate to about one-eighth or 20 millimoles per liter. This last quantity is, however, more variable. The base bound by protein may amount to about 30 millimoles per liter, since under the conditions usually existing in normal blood each molecule of protein accounts for nearly three molecules of base. The concentration of oxygen may vary from almost nothing to the above-mentioned concentration of hemoglobin; it is, therefore, always low. The total concentration of dissolved substances (ions plus molecules) in plasma, as measured by the depression of the freezing point, is approximately 300 millimoles per

liter. Since osmotic equilibrium exists and there must be, at most, very slight mechanical pressure at the surface of the red cell, this is also the concentration of cell contents. Accordingly, as a solution, blood is not much more concentrated than the dilute solutions of physical chemistry. It is certain, in view of these facts, that the components of the system which are above enumerated account for a very large part of the dissolved constituents of normal blood, whether these are measured in terms of mass or of mole-fraction. But, in disease, notably nephritis, this is sometimes not a close approximation.

Since the components, in Gibbs's sense, of the physico-chemical system blood are the most important variables with which we shall have to deal throughout this book, it will be convenient to characterize and to discuss them a little more clearly. Henceforth we shall recognize the following components of blood and, except incidentally, no others: (1) Water, H_2O. (2) Carbon dioxide, CO_2. (3) Oxygen, O_2. (4) Hydrochloric acid, HCl. (4a) The sum of all other acids except proteins (that is, excluding carbonic and hydrochloric acids and proteins) which are combined with base, HX. (5) The sum of all the bases (except protein) of the plasma (or serum), BOH_s. (6) The sum of all the bases (except protein) of the cells, BOH_c. (7) The sum of the proteins of plasma (or serum), P_s. (8) The protein of the cells, P_c or, approximately, Hb. For most purposes components 4, HCl, and 4a, HX, may be considered as a single component.

The recognition of two components P_s and P_c (or Hb) is due to two facts: the inability of proteins to cross the cell wall, and the change in the properties of the hemoglobin molecule which accompanies oxygenation. Either of these facts would make impossible the approximation which considers the proteins of blood collectively as one component. It may be noted that from the physico-chemical point of view hemoglobin is an exceptional protein. But the proteins of plasma, though their properties are unquestionably adaptive, are typical. The recognition of

BOH_s and BOH_c as two components is due to the fact, as yet unexplained, that base crosses the cell wall inappreciably or not at all.

Since blood is normally without measurable metabolic activity in many species, notably in man, it is easy to establish a state of equilibrium, or at least a stationary state at present indistinguishable from equilibrium, in this system, and to study it at leisure. This is an inestimable advantage which would alone suffice to make of blood the most favorable subject for many investigations in general physiology. It should be noted that blood of the type which has no metabolism of its own is always in equilibrium in all the vessels of the body where the walls are impermeable to its constituents. At any moment this is the case for a variable but great part of all the blood in the body.

In the physico-chemical equilibrium of blood two gaseous components, carbon dioxide and oxygen, are involved and take a large part. In accordance with the gas laws the true thermodynamical concentrations throughout the system may be calculated with a high degree of accuracy from the partial pressure in a gas phase which has been brought into equilibrium with the liquid phase. Hence, in the case of blood, gas analysis yields information of a theoretically unimpeachable nature concerning the values of two variables. It is this fact, together with the possibility of establishing a condition of equilibrium, which, even more than the relative simplicity of blood, has made possible most of the experiments with which these lectures are concerned.

Three physiological functions of blood, that of *milieu intérieur* or environment of the organs and tissues, and those of vehicle for the transport of oxygen and of carbonic acid between lungs and tissues, are describable to a first approximation in terms of the components just enumerated and of their physico-chemical interactions. Together with the transport of water and of glucose, concerning which knowledge is insufficient, and perhaps the

regulation of temperature, these are quantitatively the most considerable functions of blood. They are its only physiological functions which will be considered in this book.

As environment of the organs and tissues it is necessary that the physico-chemical properties of blood plasma should be liable to no more than small fluctuations. The properties here in question are temperature, viscosity, and a few other physical properties, and the true thermodynamical concentrations or activities of the various ions and molecules of the solution. In particular the concentration of water (osmotic pressure), commonly measured by the depression of the freezing point, the concentrations of hydrogen and hydroxyl ions, often expressed as pH, and the concentration of the bicarbonate ion HCO_3^-, are important. Not less so, though almost always subject to relatively greater fluctuations in each respiratory cycle of the blood, are the concentrations of oxygen and free carbonic acid, which, in accordance with the principle above referred to, are commonly measured by the pressures of oxygen and carbon dioxide gases in equilibrium with blood. From this point of view the degree of oxygenation of hemoglobin, except indirectly through its relation to the concentration of free oxygen, is less important.

Claude Bernard's theory of the constancy of the *milieu intérieur* was formulated at a time when information regarding the composition of the blood was scanty and when the theories of physical chemistry were still so undeveloped that an exact quantitative study of the problem was impossible. It has, however, received remarkable confirmation, especially from the discoveries and theoretical advances of the present century. Yet it must be evident that there can exist no more than a rough approximation to constancy in the properties of blood. The following changes are too well known to call for comment: (1) The transition from arterial to venous and from venous to arterial blood. (2) Diminution in the mass of cells (anemia) or the reverse (polycythemia). (3)

Diminution in the amount of base available for the transport of carbonic acid (acidosis) or the reverse (alkalosis). Changes also occur as a result of the formation of lymph, of cerebro-spinal fluid, of urine, and of other similar processes. In like manner absorption of the contents of the alimentary canal is not without effect. As a result of such phenomena every constituent of the blood must be constantly varying in concentration.

Taking these facts into account, it is plain that the theory must now be modified. As a first approximation we still say that the properties of the blood are constant, and this remains the most important proposition that can be stated about this subject. A more accurate proposition asserts that throughout the body the properties of blood are variable, but that each organ and tissue and even perhaps each portion of an open capillary contains blood of constant composition. In turn this proposition may be modified to the effect that the properties of blood are always varying, though slightly and almost inappreciably, with changing metabolism and with changing activity of the different organs, and that in pathological conditions changes may become much greater. Each of these propositions is useful, the first for general purposes and as a means of understanding the whole organism, the second in studies of the physiological changes accompanying the circulation of the blood, the last in the accurate study of special problems of physiology and pathology.

Such changes, like all other physiological processes, are in a measure adaptive. It will be one of the principal topics of later chapters to explain, with the help of accurate quantitative data, how this is true of the respiratory cycle. We shall also consider at length, from the standpoint of the theory of the constancy of the environment, the adaptive character of changes of the blood in acidosis and alkalosis, and in anemia. Other examples may be found in the case of variation of the metabolism.

Among the physico-chemical properties of blood some are more important than others. This may be, as with the

concentration of oxygen, because of the nature of metabolic processes. It may be, as in the case of the relative concentrations of sodium and potassium ions, because of the structure of the cells and of protoplasm. Or it may be because even in simple physico-chemical systems some things are more important than others. Among the variables of the last class, temperature, the concentration of water (or osmotic pressure), and the concentration of hydrogen and hydroxyl ions, are particularly notable.

Stability of hydrogen ion concentration is a property of all forms of blood and also of the waters of the earth. In higher organisms this condition is, however, insured by elaborate physiological regulatory processes. Constancy of osmotic pressure of blood is less general, but seems to have been acquired at a relatively early stage of evolution. Constancy of temperature insured by physiological regulation is peculiar to the birds and mammals. It seems probable that temperature and osmotic pressure in the higher vertebrates are approximately optimal conditions for the activities of protoplasm. In the case of the hydrogen ion concentration there can be no doubt that this is so.

It may be supposed that the properties of protoplasm were early adapted to the conditions of hydrogen ion concentration that are to be found in natural waters. In respect of osmotic pressure there seems to be an evolutionary convergence on the conditions which exist in higher animals, and, as for temperature, it is remarkable that the birds and mammals have independently evolved a temperature regulation at about 40°. In view of these facts it may be assumed that such conditions are in general favorable to the class of systems which we call protoplasm, but that individual systems belonging to this class may vary more or less as a result of special adaptations.

Total fluid pressure in blood is ordinarily unregulated, but is not commonly subject to wide variations. Nevertheless the difference in pressure between blood and the surrounding tissues is accurately regulated. In the arteries

this is of merely dynamical importance, but in the capillaries, even though the difference in pressure is small, it is a factor in the formation of lymph, of cerebro-spinal fluid, and of urine.

The properties of the components of the blood are highly adapted to the function of preserving the constancy of the environment of cells and tissues and to the functions of transporting carbonic acid and oxygen. For example, the buffer action of a mixture of carbonic acid and its salts is unsurpassed as a means of maintaining a nearly neutral reaction through *direct* neutralization of acid or base. To this process the proteins of the blood also contribute, while hemoglobin, indirectly as a result of cyclical changes attendant upon its union with oxygen in the lung and the dissociation of this compound in the tissues, largely compensates for the changes in alkalinity which would otherwise result from simultaneous changes in the carbonic acid content of the solution. Not less remarkable is the adaptation of the properties of hemoglobin to the transport of oxygen. All of these functions are performed, however, not by the blood alone, but in cooperation with the lungs, the kidneys, and other organs.

Hemoglobin, as just noted, is adapted to three functions, oxygen transport, carbonic acid transport, and regulation of the alkalinity of blood. Because thus engaged in three physiological activities, it serves to correlate them and to assure their harmonious concurrence. It is the only substance except hemocyanin which is now known to possess so many functions and thus the best example of adaptation of chemical properties to organic ends.

The eight components of blood which have been enumerated are involved in various chemical and physicochemical processes. These processes, though all interconnected, may be readily distinguished. In certain cases it is even possible to isolate them.

Water dissociates in blood to a very slight degree. Yet the law of its dissociation is important because it relates

the concentration of hydroxyl ions to that of hydrogen ions and thus permits the calculation of one from estimates of the other. Water is the solvent of all other substances with which we shall be concerned, and in every case which involves these substances there appears to be a sufficiently close approximation to the state of true solution. As a result of changes in the concentration of dissolved substances during the respiratory cycle water passes back and forth by osmosis between cells and plasma. It also reacts with carbon dioxide to form carbonic acid.

Carbon dioxide (CO_2) is not known to enter into any other chemical reaction, and we do not know whether it can pass the cell wall. Certain it is, however, that one or both of the substances carbon dioxide and carbonic acid, as well as the bicarbonate ion, appears to move freely in and out of the cell. Carbonic acid dissociates slightly into hydrogen and bicarbonate ions but, like water, exists almost entirely as undissociated molecules. These consist of both CO_2 and H_2CO_3. The concentration of bicarbonate ion is nevertheless considerable, for the bicarbonates of blood greatly exceed the free carbonic acid. These salts are highly dissociated, or, according to modern theories, perhaps completely.

The concentration of free oxygen in blood is much lower than that of free carbonic acid ($CO_2 + H_2CO_3$). Oxygen diffuses across the cell wall. It enters into a loose reversible chemical combination with hemoglobin to form oxyhemoglobin.

Hydrochloric acid is present as highly or completely dissociated salts. The chloride ion moves freely between cells and plasma. The other acids of blood (HX) behave in general like hydrochloric acid. Some of them, however, like phosphoric acid, lactic acid, and hydroxybutyric acid, are less strong. In these cases salt formation may be incomplete, in accordance with the well-known laws of equilibrium between acids and bases.

The bases, both of the plasma and of the cells, also

exist only as salts, since the concentration of hydroxyl ions is so small that the amount of free base may be regarded as infinitesimal. There is no satisfactory evidence that bases can pass across the cell wall, and it is certain that in man they do not pass in large quantities.

The proteins of plasma, as amphoteric substances of high molecular weight which contain considerable numbers of weak acid and basic groups of different strengths, are more difficult to characterize. Since the isoelectric points of these proteins lie far on the acid side of the reaction of the blood, it seems probable that their basic groups are all free and undissociated, while their acid groups are, in varying degrees, but by no means completely, ionized as salts of the plasma bases. These proteins cannot pass the cell wall.

The condition of hemoglobin in the cells is similar. In this case, however, the isoelectric point falls near the reaction of blood. It is therefore probable that the basic groups may not be quite undissociated. Nevertheless, in general, the ionization of the acid groups seems to be alone large and for many purposes we may adopt the fiction that nothing else is in question. As above stated, hemoglobin combines in widely varying amounts with oxygen. This protein also cannot pass the cell wall.

Such, in the briefest possible outline, is the condition of the components of this system. The description is but roughly approximate and it is stated in terms of theories, some of which are at present in question among physical chemists. I trust, nevertheless, that it will be found both sufficiently accurate and sufficiently complete for the present purposes.

Whenever the physiological state of the individual is not changing, the only important variations in the composition of the blood are the cyclical changes in oxygen and carbon dioxide content in the capillaries of the lungs and in those of the greater circulation. These changes are, however, attended by important modifications of chemical and physico-chemical equilibria which involve all the

other components of the blood. Even in such a stationary state, however, there are always lesser variations in the masses of the other components on account of the formation of lymph and other fluids, on account of absorption from the intestine, and for other reasons. With changes in physiological activity or changes from health to disease, variations in all the other components become important. We shall first consider the stationary state of the body and assume as a sufficiently accurate approximation, that the only variations in the blood are due to the exchanges of carbon dioxide and oxygen across the capillary walls.

When carbon dioxide enters or leaves the blood there is a disturbance of equilibrium in both plasma and cells between this substance, the bases, and the other weak acids. If for convenience we neglect the phosphates and other similar compounds included in the component HX and also the ionization of hemoglobin as a base, we may represent the process by the two reversible reactions:

$$H_2CO_3 + BP_s = BHCO_3 + HP_s,$$
$$H_2CO_3 + BP_c = BHCO_3 + HP_c,$$

and we may say that the absorption of carbonic acid in varying amounts by blood is chiefly due to the transfer of base from proteins to carbonic acid. It must be noted that the above reactions are greatly simplified schemeta, for the proteins are far from being simple monobasic acids.

When the entrance of carbonic acid into the blood, accompanied by these two reactions, takes place, there must evidently be an increase in the concentration of free carbonic acid, of bicarbonate, and of those protein molecules in which certain acid radicals are free, and a decrease of the concentration of protein molecules in which certain acid radicals are ionized as salts. Hence there result two further changes in the system: (1) An increase in the hydrogen ion concentration, and (2) an increase in the total concentration of dissolved substances. The first change depends on the fact that hydrogen ion concentration is

proportional to the ratio of free carbonic acid concentration to bicarbonate concentration,

$$(H^+) = k' \frac{(H_2CO_3)}{(BHCO_3)},$$

and that, according to the law of mass action, this ratio must always increase when carbonic acid is added to the system. The second change depends upon the fact that the number of molecules and of ions derived from the components B and P remains roughly constant, while each molecule of carbonic acid added to the system forms either an osmotically active molecule of H_2CO_3 (or CO_2), or an active ion of HCO_3^-.

In general these changes of concentration, resulting from the addition of a small quantity of carbonic acid to the system, will be of different magnitudes in cells and in plasma. This is in large measure due to the great difference in concentration of protein in the two phases, for where the concentration of protein is higher, as in the cells, more bicarbonate will be formed, while the concentrations of free carbonic acid will remain equal, or nearly so, in the two phases. The high buffer value of hemoglobin greatly enhances this disparity. A condition of equilibrium in respect of hydrogen ions and of water existed before the addition of carbonic acid, but as a result of the changes following the addition of carbon dioxide unequal changes in the concentrations of these substances must have taken place in cells and in plasma, since increase in bicarbonate is accompanied, under these circumstances, by diminution of hydrogen ion concentration and by increase of osmotic pressure. Thus there must exist a tendency for acid and for water to pass from plasma to cells in order to reëstablish the disturbed heterogeneous acid-base equilibrium and the disturbed osmotic equilibrium. In accordance with these considerations, the addition of carbonic acid to blood is, in fact, followed by a movement of water and of hydrochloric acid and other acids into the cells from the plasma.

All these changes are reversed when carbonic acid passes out of the blood. In this complicated chain of events, everything takes place in a manner that can be explained on theoretical grounds and imitated in artificial systems. But failure of the bases to take part in the osmotic process, and to move across the cell wall in an opposite direction to that taken by the acids, is as yet unexplained. This is a defect in our theories, but it is fortunately of little importance in the physiological study which we are about to undertake.

Oxygen, dissociated and free, exists in the blood in small amount. The greater part is always in chemical combination with a radical of the hemoglobin molecule in the form of oxyhemoglobin. When oxygen enters or leaves the blood, corresponding changes in the concentration of free oxygen occur, but the greatest changes are in the concentration of oxyhemoglobin.

The combination of oxygen with hemoglobin has been the subject of many investigations, but is not yet thoroughly understood. It is, however, known that, when complete, the union of oxygen with hemoglobin is in the ratio of 32 to about 16,700 by weight, that this corresponds to the presence of one molecule of oxygen for each atom of iron in the hemoglobin molecule, and, accordingly, that the simplest conceivable formula of oxyhemoglobin is HbO_2. If this were the case the reversible reaction representing the union of oxygen with hemoglobin would be

$$Hb + O_2 = HbO_2.$$

Evidence exists, however, in favor of the hypothesis that the molecular weight of hemoglobin is 67,000, each molecule containing four atoms of iron, and consisting perhaps of a union of four identical or very similar compound-protein radicals, of which each corresponds to the above-mentioned molecule of molecular weight, 16,700. On this hypothesis the formula of oxyhemoglobin would be Hb_4O_8, that of reduced hemoglobin Hb_4. For the present the mechanism of such a possible reaction must re-

main uncertain, since the intermediate forms Hb_4O_6, Hb_4O_4, Hb_4O_2 may or may not be involved in the process. In any case it is plain that the chemical equilibrium between a simple substance of low molecular weight, like oxygen, and another of very complex structure whose molecular weight is a thousand times greater may well present novel features, the elucidation of which will perhaps be difficult.

We have already noted the fact that, apart from its variable union with oxygen, the hemoglobin of blood is not always in the same state, but that its acid radicals are partially ionized as salts and that the extent of this salt formation is variable. Although, theoretically, any changes in one radical of a molecule must exert an influence on the other radicals, it was hardly to be expected on chemical grounds that, under these circumstances, the effect of one radical on the other would be sensible. This does, however, seem to be the case. Certain it is that as carbonic acid is added to blood and the ionization of the acid radicals of the hemoglobin molecule diminishes, the affinity of hemoglobin for oxygen also diminishes. This action cannot be other than reciprocal, and, in fact, as oxygen enters into combination with hemoglobin the ionization as acid seems to increase, or in other words, hemoglobin appears to become a stronger acid which displaces carbonic acid from its union with base. This is most easily explained by the hypothesis that near the point of union of oxygen in the hemoglobin molecule is an acid radical whose dissociation constant is greater when oxygen is united with the molecule, and reciprocally that when this acid radical is ionized the affinity for oxygen is increased. Apart from all questions concerning the chemical mechanism of this interaction between oxygen and carbonic acid, its physiological importance must be apparent. For, by this means, the entrance and exit of carbonic acid from the blood facilitates and is facilitated by the exit and entrance of oxygen. Also the variations of hydrogen ion concentration, small though they may be, which must

otherwise accompany the respiratory variations in carbonic acid concentration, are greatly diminished by these reciprocal changes in the acidity of hemoglobin.

Such changes in the hemoglobin molecule cannot fail also to modify the osmotic exchange between cells and plasma. This may be attributed, as above explained, to disturbances of the heterogeneous hydrogen and hydroxyl ion equilibria and to accompanying disturbances in the total concentrations of cell and plasma solutions, through variations in concentration of the bicarbonate ion in the two phases. Evidently all these factors must be modified by the peculiar behavior of the hemoglobin molecule which is now in question. Thus hemoglobin, which is the most highly adapted component of the blood, at least in respect of the respiratory function, may be regarded as a center from which all the respiratory changes radiate. It will be convenient sometimes to adopt this point of view.

It is now apparent that all the physico-chemical equilibria with which we have been concerned are parts of a single equilibrium. No one of these processes can be modified without a modification of all the others, and such modifications are always large. This was by no means to be expected on chemical grounds. For, though it is true that in a physico-chemical system each component and each reaction does exert an influence on all the others, such influences are often negligible in a first approximation.

Roughly, the changes in blood which constitute the respiratory cycle may be represented schematically as follows:

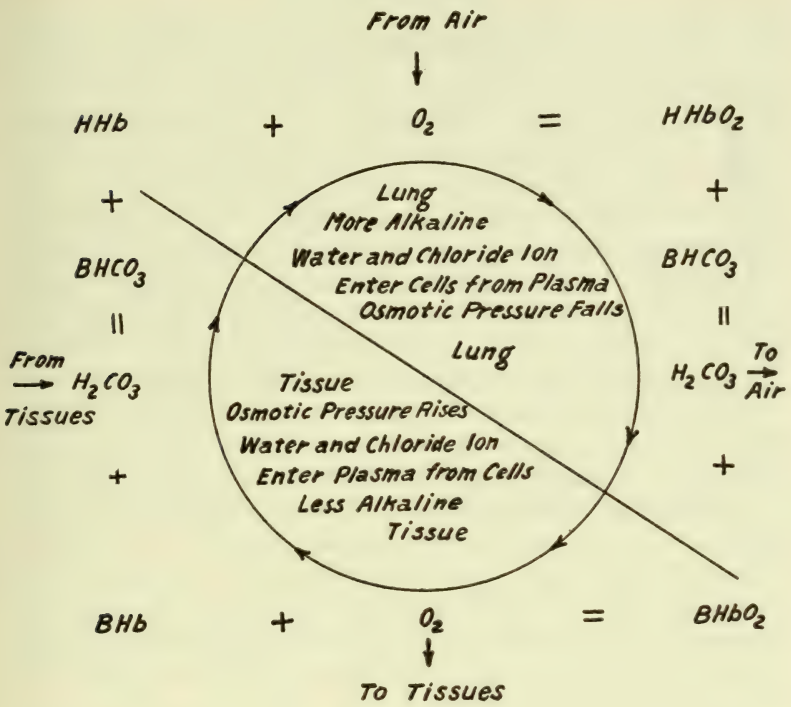


Fig. 1

The Respiratory Cycle

But as we have seen, other factors are also involved, and no diagram of this sort can adequately describe the whole process. In order to make further progress in the description of the blood, we must, therefore, pass on to a quantitative study of the facts.

CHAPTER III

ACID-BASE EQUILIBRIUM

THE quantitative description of blood that we shall now undertake necessitates a careful examination of several of the chemical equilibria referred to in the preceding chapter. Among these the most important are the equilibrium between carbonic acid and base, the equilibrium between blood proteins and base, the equilibrium between carbon dioxide and whole blood, the equilibrium between oxygen and whole blood, and the heterogeneous equilibrium between red cells and plasma.

The relation between carbonic acid and base, which is the largest factor in the whole acid-base equilibrium of blood, is responsible for many of the most characteristic properties of living things. It is not peculiar to organic fluids, but in some of its features is common to all the natural waters and aqueous solutions of the earth. In it we may discern one of the elementary properties of the environment, a primitive and, so to say, pre-organic condition which is not less important geologically than it is physiologically.[23]

The substance carbon dioxide, upon solution in water, reacts to form carbonic acid:

$$CO_2 + H_2O = H_2CO_3.$$

Unfortunately the extent of this reaction is hardly measurable.[24] The weak acid thus formed ionizes incompletely according to the reaction

$$H_2CO_3 = H^+ + HCO_3^-.$$

[23] L. J. Henderson, *The Fitness of the Environment,* chaps. IV, V, New York, 1913.

[24] Thiel and Strohecker, *Berichte der deutschen chemischen Gesellschaft,* XLVII, 945, 1061 (1914). Faurholt, *Zeitschrift für anorganische Chemie,* CXX, 85 (1921).

Applying the law of mass action we obtain the equation

$$k = \frac{(H^+)\cdot(HCO_3^-)}{(H_2CO_3)}. \qquad (1)$$

This is exact if the quantities in parentheses are taken as representing the true thermodynamical concentrations or activities of the respective substances. Also, in accordance with Henry's law, we may write the equation

$$(H_2CO_3) = \gamma_1 \cdot \lambda \cdot c \cdot (pCO_2), \qquad (2)$$

where pCO_2 represents the partial pressure in millimeters of mercury of carbon dioxide in equilibrium with a solution, c, the molal concentration of free carbonic acid in the solution when $pCO_2 = 1$, γ_1 the activity coefficient of carbonic acid, and λ that of water in the solution. If a be the absorption coefficient of carbon dioxide we have

$$c = \frac{a}{22.4 \times 760}.$$

Also, if bicarbonate be present in the solution we have

$$(HCO_3^-) = \gamma_2 \cdot [BHCO_3]. \qquad (3)$$

Here $[BHCO_3]$ as usual represents the ordinary molal concentration of bicarbonate and γ_2 the activity coefficient of the bicarbonate ion in the solution.

Combining equations 1, 2, and 3, and rearranging terms, we finally obtain an equation which exactly expresses the hydrogen ion concentration of a buffer mixture of carbonic acid and bicarbonate.

$$[H^+] = \frac{\gamma_1 \cdot \lambda \cdot k}{\gamma_2} \cdot \frac{c \cdot (pCO_2)}{[BHCO_3]}. \qquad (4)$$

The activity coefficients γ_1, γ_2, and λ are variables; their values depend upon the nature and the concentration of every substance present in the solution and for the present they cannot in general be accurately estimated. Valuable information concerning these quantities and the equilibrium in question may be found in a recent paper by

Walker, Bray, and Johnston.[25] The important similar case of the phosphate buffer system has been studied both theoretically and experimentally by Cohn.[26] At this point ignorance is fortunately less important in physiological than in physico-chemical researches. For, in the first place, a high degree of accuracy in the estimation of the absolute values of these activity coefficients is not necessary for many physiological purposes, and secondly blood plasma is perhaps even more constant as a thermodynamical environment than as the environment of cells and tissues. Therefore for blood, or more precisely speaking for blood plasma or blood corpuscles of each species, we may write the equation

$$\frac{\gamma_1}{\gamma_2} k' = k'' = \text{Const.} \tag{5}$$

From this relation we obtain the approximate expression:

$$[H^+] = k'' \cdot \lambda \cdot \frac{c \cdot (pCO_2)}{[BHCO_3]}. \tag{6}$$

$$\text{Also } k'' \cdot \lambda = k' = \text{Const.} \tag{7}$$

Therefore equation 6 is equivalent to the following equation:

$$[H^+] = k' \frac{[H_2CO_3]}{[BHCO_3]}. \tag{8}$$

And this may also be expressed logarithmically as follows:

$$pH = pk' + \log [BHCO_3] - \log [H_2CO_3]. \tag{9}$$

Here, in accordance with a custom which is sometimes bewildering because of the inverse variation, we have the definitions

$$pH = -\log [H^+],$$
$$pk' = -\log k'.$$

[25] Walker, Bray, and Johnston, *Journal of the American Chemical Society,* XLIX, 1235 (1927).

[26] E. J. Cohn, *Journal of the American Chemical Society,* XLIX, 173 (1927).

In equations 8 and 9 the term $[H_2CO_3]$ stands for the total molal concentration of free carbonic acid, whether in the form H_2CO_3 or in the form CO_2. The value of k', as above remarked, is subject to variations from one thermodynamic environment to another. In extreme cases these variations may amount to more than a fivefold increase in the value of k', but this is only true for widely differing systems. For similar systems the value of k' is always approximately constant, and there is much experimental evidence that for blood plasma the approximation is a close one.[28]

Twenty years ago, with the help of equation 8, I explained in detail the state of carbonic acid in blood plasma, its share in the acid-base equilibrium of blood, in the regulation of blood alkalinity, and the other physiological features of the behavior of this substance.[29] Since that time these questions have been the subject of a large number of investigations. As a result, the facts are widely known. Therefore, since the conclusions of my early investigations are no longer the subject of dispute, this subject may be rapidly dispatched.

The value of k' is nearly 7.58×10^{-7} ($pk' = 6.12$) in blood plasma at 37°. At this temperature we have also

$$(H^+) \cdot (OH^-) = k_{H_2O} = 2.8 \times 10^{-14}. \qquad (10)$$

Therefore a neutral solution at body temperature is one in which the following equations hold:

$$(H^+) = (OH^-) = \sqrt{2.8 \times 10^{-14}} = \frac{5}{3} \times 10^{-7}\text{N}.$$

Since $\log \frac{5}{3} \times 10^{-7} = -6.78$, neutrality is also defined by the relations

$$pH = pOH = 6.78. \qquad (11)$$

[28] Hasselbalch and Lundsgaard, *Skandinavisches Archiv der Physiologie,* XXVII, 13 (1911), and later papers by many authors.

[29] L. J. Henderson, *American Journal of Physiology,* XXI, 427 (1908); *Ergebnisse der Physiologie,* Jahrgang VIII, p. 254 (1909).

Accordingly, the condition for an acid reaction at 37° is given by the inequalities

$$(H^+) > \frac{5}{3} \times 10^{-7}N > (OH^-), \tag{12}$$

$$pH < 6.78 < pOH, \tag{13}$$

and the condition for an alkaline reaction by the inequalities

$$(H^+) < \frac{5}{3} \times 10^{-7}N < (OH^-), \tag{14}$$

$$pH > 6.78 > pOH. \tag{15}$$

It is evident (equation 8) that in a solution having the properties of blood plasma, when the concentrations of free carbonic acid and of bicarbonate are equal, the value of the hydrogen ion concentration must be the same as that of k', *i.e.*, $(H^+) = 7.58 \times 10^{-7}N$; the value of pH the same as that of pk', *i.e.*, $pH = 6.12$. This is an acid reaction for which the concentration of hydrogen ions is nearly five times as great as at the neutral point. About this value of the hydrogen ion concentration the values

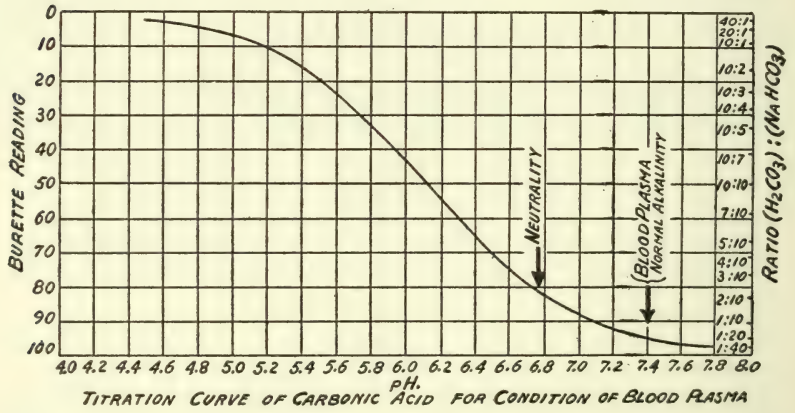


Fig. 2

Approximate Description of Buffer Action

for solutions containing mixtures of carbonic acid and bicarbonate vary symmetrically as the relative concentrations of the two substances change. Thus if the ratio be 1:2 the hydrogen ion concentration becomes 3.79×10^{-7}N; if the ratio be 2:1, 15.2×10^{-7}N; if the ratio be 1:10, 7.58×10^{-8}N; if the ratio be 10:1, 7.58×10^{-6}N.

In figure 2, corresponding to the well-known titration curves of physical chemistry, these conditions are completely illustrated. For convenience hydrogen ion concentration is represented logarithmically by values of pH as abscissas. The ordinates represent the readings of a burette from which sodium hydroxide solution is being delivered to a solution originally containing free carbonic acid but no bicarbonate, which is assumed to possess and to preserve the properties of blood plasma. Values of the ratio $\frac{[H_2CO_3]}{[BHCO_3]}$ are also represented as ordinates. While this curve is quite as accurate a representative of the facts of the equilibrium in blood plasma as is now possible, and probably very accurate indeed, it must be borne in mind that in a more simple solution, such as a pure solution of carbonic acid, the value of k' is bound to vary sensibly on account of variation in the activity coefficients of the substances involved in the reaction. Such variations will depend especially upon changes of the ionic strength of the solution. In such cases the curve suffers systematic deformation. The nature of the case in solutions of phosphates has been completely worked out in the above-mentioned memoir of Cohn,[30] who has shown that the Debye-Hückel theory of ionization may be successfully applied.

In order to illustrate the departure from the simple approximate equation of acid-base equilibrium (equation 8) which is to be expected when the conditions are not constant, as they are in blood plasma, and are subject to the

[30] E. J. Cohn, *Journal of the American Chemical Society,* XLIX, 173 (1927).

variations which commonly arise in pure solutions, figure 3, taken from Cohn's paper,[31] is printed. It is easy to see that the form of any moderately short portion of any of the curves of this figure is approximately identical with that of the corresponding portion of the simple curve of figure 2, but that over wider ranges there is an important discrepancy. Also, the positions of the curves, corresponding to the values of k', are variable.

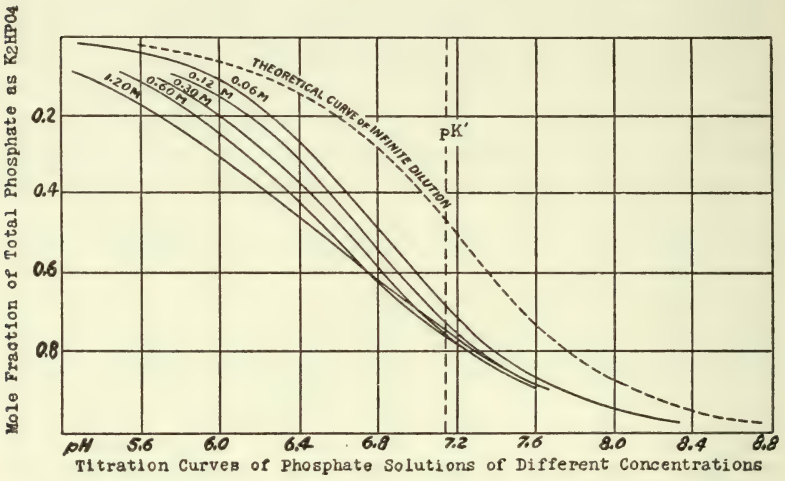


Fig. 3

Accurate Description of Buffer Action

The buffer value of carbonic acid may be conceived as the resistance of a solution containing carbonic acid and bicarbonate (and in certain cases carbonate) to a change of hydrogen ion concentration. Speaking more precisely, it is measured by the rate of change in such a solution of the concentration of neutralized base with change in the value of pH and this is measured by the slope of the titration curve. Obviously this is greatest, the curve being steepest, when the ratio of free acid to salt is equal to 1.

[31] *Loc. cit.*, p. 180.

It diminishes symmetrically in either direction from this middle point, which is a point of inflection. Therefore in blood the homogeneous buffer action of carbonic acid is a maximum when $(H^+) = 7.58 \times 10^{-7}$N. It is less at the neutral point, much less at normal blood reaction. The fact remains to be noted that precisely the same curve is obtained, but reversed, if starting with sodium bicarbonate a strong acid be added and the escape of carbon dioxide prevented.

All the other weak acids and bases of the blood behave similarly. The only difference is in the value of the characteristic constant k', the effect of variation in this quantity being to bring about translation of the curve so that its middle point falls on the abscissa corresponding to that value of the quantity pH, which for the substance in question is equal to the value of pk'. Hence under *corresponding* conditions all weak acids and bases have the same buffer value. For the case of weak bases, however, the term pH must be replaced in the equation by the term pOH and the ratio of acid concentration to salt concentration by the ratio of base concentration to salt concentration.

It is evident that an acid slightly weaker than carbonic acid, for instance mono-sodium phosphate, would be more effective than carbonic acid in preserving a precisely neutral hydrogen ion concentration by means of a homogeneous reaction of the nature of buffer action in a system having the properties of blood plasma. Moreover, since the reaction of blood is faintly alkaline, a still weaker acid would be in this respect still more efficient. But the buffer action of carbonic acid in blood is not due to a homogeneous chemical equilibrium, for carbonic acid escapes into the alveolar air.

When free carbonic acid is in equilibrium with carbon dioxide in the air, the conditions become very different from those in the homogeneous buffer system.[32] We may

[32] L. J. Henderson, *Ergebnisse der Physiologie,* Jahrgang VIII, p. 301 (1909).

first consider the case of constant partial pressure of carbon dioxide in the gas phase, say $pCO_2 = 40$ mm., which corresponds to the ordinary conditions in the human lung. Adopting the customary physiological measure of concentration of carbonic acid in volumes per cent, we then have for average plasma

$$\begin{aligned}[H_2CO_3] &= 0.0687 \times (pCO_2),\\ &= 0.0687 \times 40,\\ &= 2.75 \text{ vols. per cent,}\end{aligned}$$

and if the pressure of carbon dioxide in the air remains constant and equilibrium between air and solution persists, then the concentration of free carbonic acid in the solution must remain constant at the value 2.75 volumes per cent. Let us consider the changes that will occur during the titration of such a solution, which is assumed to

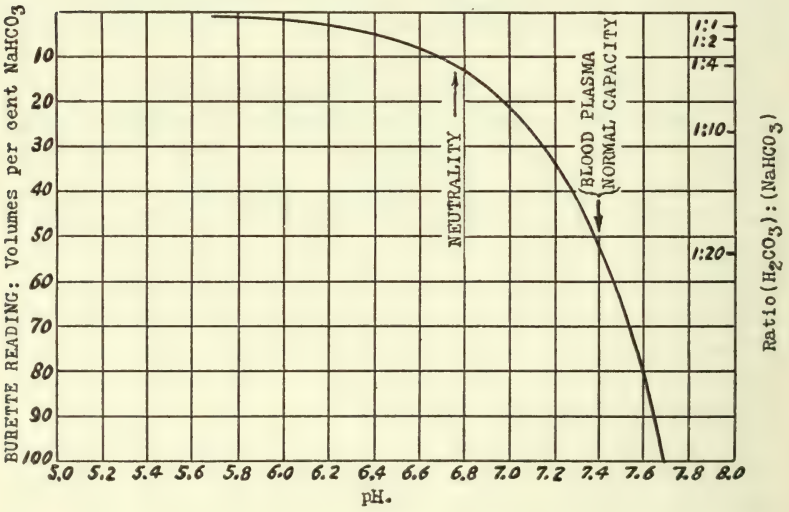


Fig. 4

Heterogeneous Buffer Action

possess the properties of blood plasma. In this case equation 7 becomes

$$[H^+] = 7.58 \times 10^{-7} \times \frac{2.75}{[BHCO_3]},$$

$$\text{or} \qquad [H^+] \cdot [BHCO_3] = 20.85 \times 10^{-7},$$

and the course of events, as a solution of sodium hydroxide is added, may be represented by figure 4. Manifestly this curve becomes steeper as alkalinity increases. It is already very steep at the neutral point, but still more so at the reaction of blood. Under these conditions the stabilizing effect of carbonic acid upon hydrogen ion concentration is very greatly enhanced. Sea water, with a hydrogen ion concentration in the neighborhood of 1×10^{-8}N, which is constantly exchanging carbon dioxide with air under conditions that preclude equilibration, while always tending in that direction, is thus stabilized in hydrogen ion concentration.[33]

The curve of figure 4 must not be extended indefinitely into ranges where the values of the ratio of acid to salt are very small, for in due course the formation of normal carbonates such as Na_2CO_3 becomes first measurable and then important. But in the organism this complication may be, for the present purposes, left out of account. In sea water, however, the relative concentration of normal carbonate is considerable.

In blood there are further complications of a physiological character. One of these is a variation of breathing following variation of the acid-base equilibrium of blood. Under ordinary conditions a close approximation to carbon dioxide equilibrium exists between arterial blood and alveolar air (pCO_2 [Blood] $-$ pCO_2 [Air] < 1 mm.).

This equilibrium persists even with wide fluctuations in the rate of movement of air through the lung. But the greater the volume of air breathed, the lower is the pressure of carbon dioxide at which the equilibrium becomes

[33] Henderson and Cohn, *Proceedings of the National Academy of Sciences,* II, 618 (1916).

adjusted. In acid intoxication, during excessive muscular work, for a period just following such work, and under other circumstances when neutralization of acid has reduced the concentration of bicarbonate in blood, increased breathing brings about a parallel reduction in pressure of carbon dioxide, and therefore in concentration of free carbonic acid in the blood. Thus by physiological control of the heterogeneous equilibrium between blood and air, the hydrogen ion concentration of blood may be kept constant and is ordinarily kept very nearly so. For example, while in the moribund the normal alkaline reaction of blood plasma (pH = 7.4) corresponding to a ratio of free carbonic acid concentration to bicarbonate concentration of about 1 : 20 may give place to a nearly neutral reaction (pH = 7.0) corresponding to a ratio of 1 : 8, in a healthy man during severe exercise the change will be much less (pH = 7.3) and the ratio will be about 1 : 16.

The effects of the homogeneous equilibrium, of the heterogeneous equilibrium, and of the physiological control of breathing may be conveniently illustrated as follows: Given an equilibrated solution of carbonic acid and bicarbonate for which $k' = 7.6 \times 10^{-7}$, in which the ratio of free carbonic acid to bicarbonate is 1 :20 and therefore the hydrogen ion concentration 3.8×10^{-8}N, let an amount of hydrochloric acid equivalent to one-half the amount of dissolved bicarbonate be added. The following chemical reaction will take place:

$$BHCO_3 + HCl = H_2CO_3 + BCl.$$

If no carbon dioxide is allowed to escape, the ratio of carbonic acid concentration to bicarbonate concentration will therefore become 11 : 10 and the hydrogen ion concentration 8.4×10^{-7}N. This represents the homogeneous buffer action of the system. Now let equilibrium be reëstablished with the atmosphere with which the system was originally in equilibrium. Then the amount of free carbonic acid in the solution will fall, in relative magnitude, from 11 to 1, the value of the ratio of the concentration of acid to that

of salt will become 1:10 and the value of the hydrogen ion concentration 7.6×10^{-8}N. This second stage corresponds to the heterogeneous buffer action of the system. Finally let carbon dioxide escape from the system until the pressure of carbon dioxide is reduced to one-half its former value. Then the ratio of acid to salt concentration in the solution will become 0.5:10 or 1:20 and the hydrogen ion concentration will return to its original value of 3.8×10^{-7}N. This corresponds to the effect of the physiological control of breathing.

Under these circumstances the readjustment of the hydrogen ion concentration has been accomplished, but the carbonic acid content of the solution has fallen to one-half its original value. Such a diminution, or an even greater one, in the carbonic acid of blood is common under pathological conditions, though at this level the hydrogen ion concentration is rarely entirely "compensated" or restored to normal. It therefore remains to build up once more a normal concentration of bicarbonate and with it of free carbonic acid. This is brought about by the excretion of salts of ammonia in the urine, while an equivalent amount of base is retained as bicarbonate, and by the simultaneous excretion of acid phosphate in place of the predominant alkaline phosphate of the blood, a heterogeneous buffer action of the phosphates which also economizes fixed base. In man the end products of metabolism are chiefly acid and the process of regulation of the acid-base equilibrium of blood through urine formation is therefore continuous.

These regulatory activities of lungs and kidneys raise an interesting physiological question which, in the case of the lung, has been much discussed. As we have just seen, it is the changing ventilation of the lung that most directly controls the acid-base equilibrium of blood. But how is the ventilation of the lung controlled? Manifestly by varying activity of the respiratory muscles. And how is this muscular activity controlled? By varying stimuli from the respiratory center in the medulla. And how are

these stimuli controlled? By the varying acid-base equilibrium of the blood. So much seems clear, and we thus have a good example of an organic regulatory process, since variation in a property of the organism has given rise to a complex cycle of events which ends in the reversal of the original variation. But closer scrutiny leads to difficulty, possibly because this scrutiny has been followed by asking the wrong question. It has been in fact customary to inquire what is the stimulus of respiration that acts upon the respiratory center. Miescher first answered that the stimulus is carbonic acid.[34] This view was supported, to a certain extent, by Geppert and Zuntz[35] and also by Haldane and Priestly.[36] Later, however, Boycott and Haldane[37] reached the conclusion that the respiratory center responds to the combined effects of carbonic acid and other acids on the reaction of the blood.

When the theory of acid-base equilibrium had been elaborated in the manner above expounded, it became clear that no choice was possible between a number of hypotheses. Accordingly, I pointed out that since the concentrations of free carbonic acid, of the hydrogen ion, and of the hydroxyl ion are not independent variables, any one of them might be the stimulus of respiration, but that the action of blood upon the respiratory center might also take place through a complicated heterogeneous equilibrium.[38]

The theory that the hydrogen ion is the stimulus of respiration was then taken up by Winterstein[39] and later by Hasselbalch[40] who showed that under certain conditions the facts are consistent with this theory. There can,

[34] F. Miescher-Rüsch, *Archiv für (Anatomie und) Physiologie,* p. 355 (1885).

[35] Geppert and Zuntz, *Pflüger's Archiv,* XLII, 195, 209 (1888).

[36] Haldane and Priestly, *Journal of Physiology,* XXXII, 225 (1905).

[37] Boycott and Haldane, *Journal of Physiology,* XXVII, 365 (1908).

[38] L. J. Henderson, *Ergebnisse der Physiologie,* Jahrgang VIII, p. 318 (1909).

[39] H. Winterstein, *Pflügers Archiv,* CXXXVIII, 167 (1911).

[40] K. A. Hasselbalch, *Biochemische Zeitschrift,* XLVII, 403 (1912).

indeed, be no doubt that as a rule the hydrogen ion concentration of blood is regulated with truly extraordinary accuracy in the manner above described, through control of the partial pressure of carbon dioxide in alveolar air.

But it is certain that other factors beside the hydrogen ion concentration, for example, body temperature or oxygen want are sometimes involved in the control of breathing, and lately Gesell[41] has returned to the theory of a complex heterogeneous equilibrium between the protoplasm of the cells of the respiratory center and the blood. Naturally such a theory involves both experimental and theoretical difficulties, but though still inadequate, it seems to be more in agreement with the description of the properties of blood which it is the object of these lectures to present, and I think also more consistent with the general characteristics of physiological processes. The theory which assumes a single stimulus seems today too simple; it assumes a process lacking in flexibility, which overlooks or at least seems to disregard the dependence of breathing upon a great many factors, and the organic harmony which subsists among them. In short, if I am not mistaken, it suffers from a defect that Haldane,[42] first among our contemporaries, has denounced with eloquence and perspicacity. I cannot resist the temptation to apply to his theory his own criticism, and to suggest that perhaps after all there is no stimulus of respiration.

In the absence of other weak acids or bases, a system containing carbonic acid and bicarbonate is incapable of considerable variation in total carbonic acid content, even when the partial pressure of carbon dioxide is allowed to vary widely. Indeed, only the free carbonic acid of the solution varies sensibly under these circumstances unless the pressure of carbon dioxide falls very low indeed, and meantime the bicarbonate concentration may be regarded as constant. Such is the condition, approximately, of the natural waters of the earth, though here, on account of

[41] R. Gesell, *Physiological Reviews,* V, 551 (1925).

[42] J. S. Haldane, *Mechanism, Life and Personality,* London, 1914.

the very small partial pressure of carbon dioxide, the following buffer reaction cannot be neglected:

$$H_2CO_3 + B_2CO_3 \rightleftharpoons 2BHCO_3.$$

But in blood, which is a vehicle for the transport of carbonic acid, conditions are less simple. In this case the proteins, which are also weak acids, share a certain portion of the base of the blood with carbonic acid. Therefore as carbon dioxide pressure increases and diminishes in blood, the bicarbonate of blood increases and diminishes, while the base bound by protein diminishes and increases. This is an example of the class of phenomena known as the partition of strong base between two (or more) weak acids.

The simplest important case of this class of phenomena occurring in the organism is the equilibrium between phosphates and bicarbonates, which was long ago studied by Black and myself.[43] The conditions of equilibrium in this system may be easily explained. Let there be present in solution the four substances carbonic acid, sodium bicarbonate, monosodium phosphate, and disodium phosphate. We have as before, assuming the conditions to be those of blood plasma,

$$[H^+] = 7.6 \times 10^{-7} \times \frac{[H_2CO_3]}{[NaHCO_3]},$$

and similarly $[H^+] = k'_{H_2PO_4} \times \frac{[NaH_2PO_4]}{[Na_2HPO_4]}.$

therefore $$7.6 \times 10^{-7} \times \frac{[H_2CO_3]}{[NaHCO_3]} = k'_{H_2PO_4} \times \frac{[NaH_2PO_4]}{[Na_2HPO_4]},$$

and $$\frac{\frac{[H_2CO_3]}{[NaHCO_3]}}{\frac{[NaH_2PO_4]}{[Na_2HPO_4]}} = \frac{k'_{H_2PO_4}}{7.6 \times 10^{-7}} = \text{Const.}$$

In dilute solution at body temperature Black and I found for the value of this ratio 0.3, whence $k'_{H_2PO_4^-} =$

[43] Henderson and Black, *American Journal of Physiology,* XVIII, 250 (1907); XXI, 420 (1908).

2.3×10^{-7}. As the ionic strength of the solution varies, this ratio will not remain constant, but will be affected with a small variation which is due to the unequal changes in the activity coefficients included in the two k' terms. The work of Cohn,[44] already mentioned, takes account of these variations for simple phosphate buffer systems at 18°, but does not extend beyond these to body temperature. The work of Black and myself,[45] on the other hand, was too early to take account of the developments of the theory of solution of the past twenty years. And neither these investigations nor those of Sørensen[46] and of Michaelis and Krüger[47] define the special conditions peculiar to blood and protoplasm. However, the value $k' = 2.3 \times 10^{-7}$ for blood at 37° is certainly a close approximation to the exact value, and, so far as it is possible to judge, is consistent with the results of all the above-mentioned investigators. In view of these considerations we may make use of the relations above defined as a means to explain a simple case of the kind of reaction which makes possible the transport of large quantities of carbonic acid in blood through absorption and dissociation, to use the conventional physiological terms. This is the more desirable because the reaction constitutes an elementary ionic equilibrium of protoplasm.[48] It is relatively inconspicuous in blood, since most blood contains only small amounts of phosphate, but is otherwise one of the important reactions of general physiology.

After all, the highest accuracy is perhaps not very important in a discussion of this question, for the general form of the functions involved in the equilibrium is more

[44] E. J. Cohn, *Journal of the American Chemical Society,* XLIX, 173 (1927).

[45] Henderson and Black, *American Journal of Physiology,* XXI, 420 (1908).

[46] S. P. L. Sørensen, *Biochemische Zeitschrift,* XXI, 131 (1909); XXII, 352 (1909).

[47] Michaelis and Krüger, *Biochemische Zeitschrift,* CXIX, 307 (1921).

[48] L. J. Henderson, *American Journal of Physiology,* XXI, 427 (1908).

to the point than the exact values of certain variables. No doubt it is desirable to know these as accurately as possible, but during the past twenty years, while estimates of these values have constantly varied, conclusions based upon the more general characteristics of the phenomena have remained unshaken. As an illustration of these characteristics we may consider the equilibrium of an aqueous solution in which the conditions of ionization resemble those of blood, in which there are present carbonic acid, phosphoric acid, and sodium hydroxide, in which there is an exchange of carbon dioxide with an atmosphere where the partial pressure of carbon dioxide is variable, and in which finally, while the concentration of carbonic acid is variable, there are precisely 1.9 moles of sodium hydroxide per mole of phosphoric acid. A simple computation founded upon the above-discussed relations and constants yields results which are graphically represented in figure

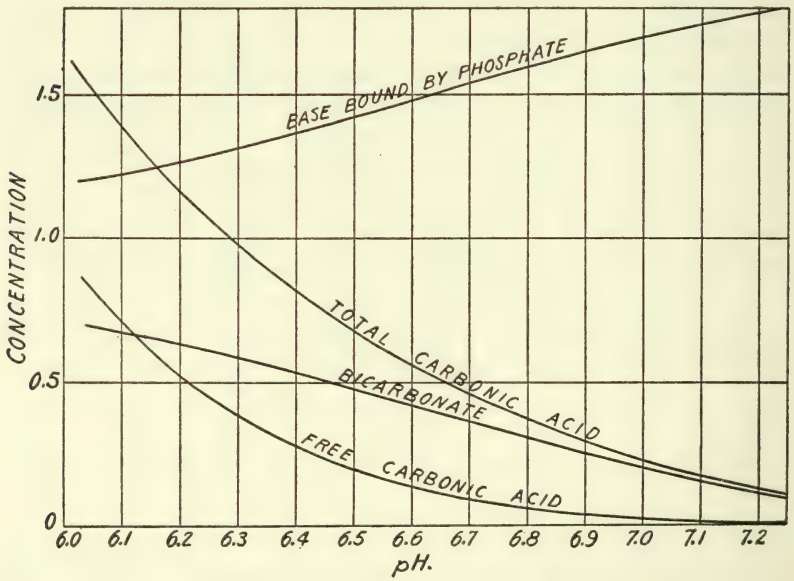


Fig. 5

Buffer Action of Bicarbonates and Phosphates

5. Here the relative amounts of base bound by phosphoric acid and by carbonic acid, and the relative amounts of free carbonic acid in the solution are presented as functions of the variable pH. These variations all depend upon variations in the partial pressure of carbon dioxide in the atmosphere, just as the conditions in arterial blood depend upon variations of the partial pressure of carbon dioxide in alveolar air. The pressures of carbon dioxide are proportional to the concentrations of free carbonic acid in the solution.

Detailed discussion of the facts represented by figure 5 is hardly necessary. Suffice it to point out, firstly, that here the concentrations of both free and combined carbonic acid vary widely with variations in the pressure of carbon dioxide and that, as the reaction of blood is approached, large variations in the concentration of combined carbonic acid accompany small variations in the partial pressure of the gas; secondly, that the buffer action of the system is the sum of the buffer actions of the carbonates and of the phosphates; and finally that the hydrogen ion concentrations at which the changes are most marked are determined by the values of k', not only for carbonic acid, but also for the ion $H_2PO_4^-$. With another weak acid for which the value of k' is different the changes must take place at a different hydrogen ion concentration, but in a similar manner. It will also be readily perceived that with varying amounts of alkali and of phosphoric acid the conditions will change in detail, while preserving their general characteristics.

These conclusions are all implicit in the equations

$$[H^+] = 7.6 \times 10^{-7} \frac{[H_2CO_3]}{[NaHCO_3]} = 2.3 \times 10^{-7} \frac{[NaH_2PO_4]}{[Na_2HPO_4]},$$

which, with slight differences in the values of k', I originally employed in working out the relations, and it may seem trivial or fastidious to spend so much time on such mere implications of a simple law. Yet nothing is more dangerous than failure to take account of the most obvi-

ous implications of general propositions, and nothing is easier. Especially in physiology, where the particular characteristics of physical and chemical phenomena often assume general importance, this should not be forgotten. Chemically how insignificant are those properties which give physiological importance to adrenaline and thyroxin, to morphine and alcohol; how seemingly trivial a physical fact is the solubility of carbon dioxide in water!

In order to facilitate the application to the problems of the physiology of blood of these considerations of the acid-base equilibrium between carbonic acid and another

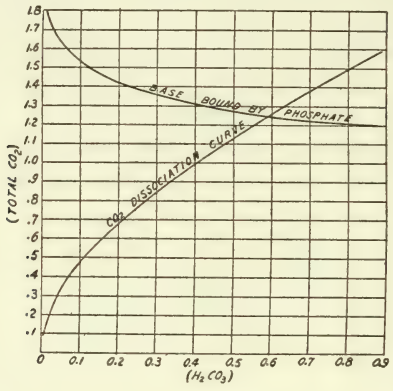


Fig. 6

Carbon Dioxide Dissociation Curve of a Simple Solution

weak acid in a heterogeneous system, the relations between free and total carbonic acid, together with the accompanying changes in base bound by phosphate are given as the two curves of figure 6. Here, as with the ordinates of figure 5, both abscissas and ordinates represent relative concentrations. The first mentioned of these two curves is of the variety known to physiologists as a carbon dioxide dissociation curve. It will be readily recognized as similar to the carbon dioxide dissociation curve of blood, such differences as exist being due to differences in concentration, to the fact that the proteins are weaker

acids than the ion $H_2PO_4^-$, and to further complications owing to the fact that several acid radicals of the blood proteins are simultaneously engaged in the process.

In sum we have now reached the conclusion that the study of the simple equation,

$$[H^+] = k' \frac{[H_2CO_3]}{[BHCO_3]},$$

which gives in a close approximation the facts concerning the state of carbonic acid and its salts in blood, is capable of explaining many features of general and respiratory physiology. Among these are the homogeneous and heterogeneous buffer action of carbonic acid, the physiological regulation of the alkalinity of blood through the activity of lungs and kidneys, and some of the features of acidosis. When the same approximation is also applied to other weak acids, we are enabled to understand the nature of the process by which carbonic acid is absorbed by blood in the tissues and given off in the lungs. This description of the facts, a quantitative one, also enables us to estimate the efficiency of carbonic acid in some of these physiological functions, and to draw the conclusions that for such purposes its properties are, on the whole, unsurpassed and almost unequalled by any other substance.[49] Nevertheless it would be an error to suppose that we have already exhaustively analyzed all features of these physiological functions. Not only are such activities far more intricate in their ramifications than the present discussion suggests, but in the conditions of equilibrium between red cells and plasma, and of the peculiar activity of hemoglobin there may be recognized laws of which we have not yet taken account. Moreover the specific properties of the acid-base equilibrium of the proteins also have to be studied if we are to arrive at an exact estimate of the conditions in blood. Accordingly we must now pass on to the proteins of blood and to the dissociation curves of the physiologists.

[49] L. J. Henderson, *American Journal of Physiology,* XXI, 173 (1908).

CHAPTER IV

DISSOCIATION CURVES

PHYSIOLOGISTS have long believed[50] that the proteins of blood combine with base, that the quantity of base thus bound is variable, and that this combination takes part in the transport of carbonic acid, through changes in the equilibrium of the reaction,

$$BP + H_2CO_3 \rightleftharpoons HP + BHCO_3.$$

Until modern physico-chemical methods could be applied to the study of this problem and the results interpreted by means of the theory of acid-base equilibrium, it remained, nevertheless, impossible to determine the facts quantitatively, and thus to understand the process clearly. In particular, since proteins are likewise able, under suitable conditions, to form dissociable salts (*i.e.,* bicarbonates) with carbonic acid, it was long uncertain how far this reaction also might take part in the absorption and escape of carbonic acid. And there were many other difficulties. At length the necessary measurements have been made, the data interpreted, and a close approximation to an exact description of the rôle of the proteins may now be undertaken.

Unlike solutions of carbonic and phosphoric acids, under the conditions existing in blood each molecule of the blood proteins possesses several acid radicals which are of such strength that they are incompletely ionized.[51]

[50] N. Zuntz, *Hermann's Handbuch der Physiologie,* vol. IV, pt. 2, p. 65 (1882).

[51] T. B. Osborne, *The Vegetable Proteins,* 2d ed., chap. V, London, 1924.

These radicals therefore take part in the acid-base equilibrium of blood and compete with carbonic acid for base.

In approaching the study of this question, we may first consider the case of a number of simple monobasic acids, HA_I, HA_{II}, . . . HA_N, all of which obey the law of acid-base equilibrium in blood discussed in the last chapter. Here we shall have

$$[H^+] = k'_1 \frac{[HA_I]}{[BA_I]} = k'_2 \frac{[HA_{II}]}{[BA_{II}]} = \ . \ . \ . = k'_N \frac{[HA_N]}{[BA_N]}. \quad (1)$$

Let there be five such acids, all present in a solution in equal concentration, so as to correspond with the case of a polybasic protein, and let the k' values be 8×10^{-8}, 4×10^{-8}, 2×10^{-8}, 1×10^{-8}, and 5×10^{-9}. Then the titration curve for the solution may be calculated. The results of this calculation are given in table 1, which includes values of pH, of $[H^+]$, of $R = \frac{[HA]}{[BA]}$ and of $[BA]$ for each acid and of $\Sigma[BA]$, which is the burette reading for each stage of the titration.

TABLE 1.

pH	(H^+)	R_1	R_2	R_3	R_4	R_5	BA_1	BA_2	BA_3	BA_4	BA_5	ΣBA	ΔBA
6.796	1.6×10^{-7}	2	4	8	16	32	.333	.200	.111	.059	.030	.733	
7.097	8×10^{-8}	1	2	4	8	16	.500	.333	.200	.111	.059	1.203	.47
7.398	4×10^{-8}	1/2	1	2	4	8	.667	.500	.333	.200	.111	1.811	.60
7.699	2×10^{-8}	1/4	1/2	1	2	4	.800	.667	.500	.333	.200	2.500	.69
8.000	1×10^{-8}	1/8	1/4	1/2	1	2	.889	.800	.667	.500	.333	3.189	.69
8.301	5×10^{-9}	1/16	1/8	1/4	1/2	1	.941	.889	.800	.667	.500	3.797	.60
8.602	2.5×10^{-9}	1/32	1/16	1/8	1/4	1/2	.970	.941	.889	.800	.667	4.267	.47

The corresponding titration curve is drawn on figure 7. For comparison the calculated course of the titration of an acid solution of equal total acid concentration, but con-

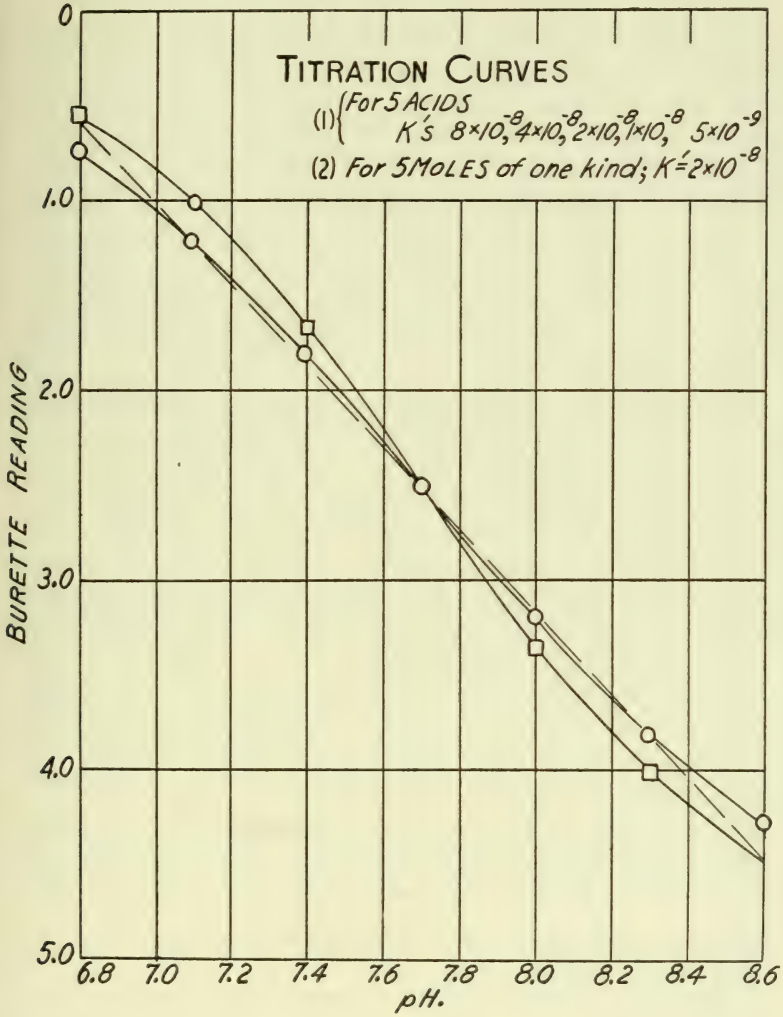


Fig. 7

Titration Curves of Mixtures and Single Acids

taining only one acid, for which $k' = 2 \times 10^{-8}$, is given. It will be seen that the characteristic S-shape of the latter curve is much modified in the titration curve of the mixture of acids. This curve, throughout a wide range of values of pH, roughly approximates to a straight line.

In general such composite titration curves, representing the overlapping buffer actions of several acid radicals, are relatively straight and often very nearly so, through a considerable range of hydrogen ion concentration. They possess minor sinuosities, according to the number and magnitudes of the different values of k' which are involved. But under favorable circumstances and for ranges of hydrogen ion concentration that are not very large, say $\Delta pH < 1.5$, a satisfactory approximation to a linear relation between values of $\Sigma[BA]$ and values of pH may be often expected.

As conditions become more complex, the validity of the simplifying assumptions which are implicit in the present theoretical discussions becomes increasingly uncertain.[52] Accordingly the application of this last result to the polybasic protein molecule presents theoretical difficulties. Nevertheless it is a fact that the titration curves of proteins, as experimentally determined, possess the characteristics that have just been described. As an example of the experimentally determined behavior of a protein we may take measurements of the titration curve of serum albumin.[53] Throughout a range of reaction equal to that represented by figure 7 the titration curve is nearly a straight line.

The behavior of the proteins of blood as acids binding base has been measured by Van Slyke, Wu, and McLean[54] and by Hastings and Harington.[55] They find that within

[52] E. J. Cohn, *Physiological Reviews,* V, 376 (1925).

[53] *Ibid.,* p. 380 (1925).

[54] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 765 (1923).

[55] Hastings, Van Slyke, Neill, Heidelberger, and Harington, *Journal of Biological Chemistry,* LX, 89 (1924).

the ranges of hydrogen ion concentration which have to be taken into account in physiological studies $\Sigma[BA]$ is a nearly linear function of pH for the serum proteins, for hemoglobin, and for oxyhemoglobin. The properties of

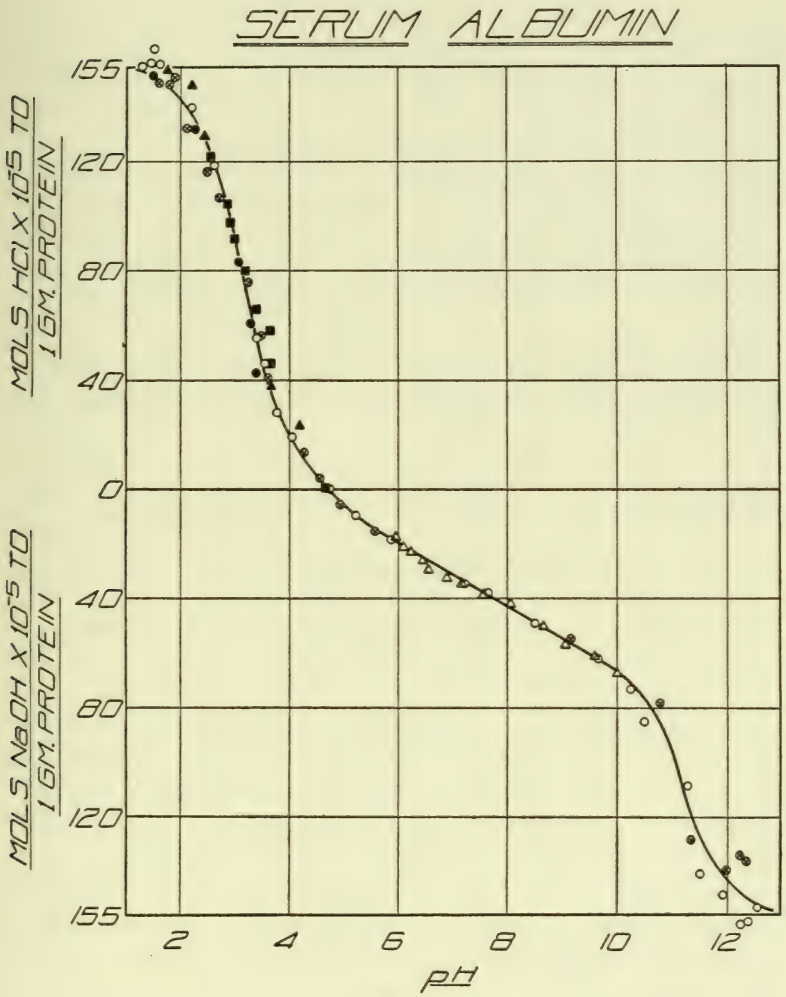


Fig. 8

Titration Curve of Serum Albumin

these three substances are expressed by the following equations:

$$[BP]_s = 0.068\,[P]_s\,(pH - 4.80), \tag{2}$$

$$[BP]_{HbO_2} = 0.216\,[HbO_2]\,(pH - 6.60), \tag{3}$$

$$[BP]_{Hb} = 0.200\,[Hb]\,(pH - 6.74). \tag{4}$$

Here the BP terms represent millimoles of base bound by serum proteins, oxyhemoglobin, and reduced hemoglobin, $[P]_s$ stands for concentration, in grams, of serum proteins and $[HbO_2]$ and [Hb] for concentrations, in grams, of oxyhemoglobin and of reduced hemoglobin. Obviously the constants 4.80, 6.60, and 6.74 represent the values of pH at which the proteins bind no base (roughly the isoelectric points).[56] The other three numerical constants are related to the values of k', but also to the number of acid radicals involved in the neutralization of base. These numbers measure the buffer values, per gram of protein, for the three cases, since

$$\frac{d\,[BP]_s}{dpH} = 0.068\,[P]_s, \tag{5}$$

$$\frac{d\,[BP]_{HbO_2}}{dpH} = 0.216\,[HbO_2], \tag{6}$$

$$\frac{d\,[BP]_{Hb}}{dpH} = 0.200\,[Hb]. \tag{7}$$

It will be seen that the buffer values per gram of the hemoglobins are about three times as great as those of serum proteins. Since normal human blood contains about three times as much hemoglobin as serum protein, it follows that the buffer value of hemoglobin in blood is almost ten times as great as that of the serum proteins. This deduction is confirmed by the experiments of Henderson, Bock, Field, and Stoddard[57] and by many later

[56] E. J. Cohn, *Physiological Reviews,* V, 395 (1925).

[57] Henderson, Bock, Field, and Stoddard, *Journal of Biological Chemistry,* LIX, 379 (1924).

studies all of which involve measurements of the properties of whole blood, of red cells, and of plasma, and are therefore independent of the above estimates. In the particular specimen of blood employed for our earliest experiments the buffer value of the hemoglobin of the blood was between eight and nine times as great as that of the serum proteins. This ratio varies with variation of the ratio of $P_c : P_s$. The actual buffer changes of the respiratory cycle also depend, as we shall see, upon the peculiarities of the heterogeneous equilibrium between cells and plasma. This buffer action of hemoglobin, within the physiological ranges of hydrogen ion concentration, is not only greater than that of the serum proteins but also exceptional among proteins in general. It is to be regarded as a well-marked adaptation.

Equations 3, 4, 6, and 7 show that the acid properties of oxyhemoglobin and of reduced hemoglobin are not identical. We shall return to the consideration of this fact.

Only on the acid side of its isoelectric point does a protein combine with large quantities of acid. As equations 2, 3, and 4 show, these points fall for serum proteins, oxyhemoglobin, and reduced hemoglobin near the pH values 4.80, 6.60, and 6.74 respectively. Therefore the union of carbonic acid with the proteins of blood is certainly small. In the case of hemoglobin it is, however, probably not negligibly small, for at the isoelectric point this substance, whether in the reduced or the oxygenated state, seems to bind measurable amounts of both base and acid. Therefore there probably exists in blood a small amount of hemoglobin bicarbonate and, of course, a larger amount of the chloride of hemoglobin. But since, for many purposes, union of acid with a basic radical for which $pk' = a$ is equivalent to union of base with an acid radical for which $pk' = 14 - a$, we may neglect this complication.

We shall now undertake a detailed consideration of the equilibrium between base, the proteins of blood, and carbonic acid. It will be convenient to begin with the equi-

librium between base, serum proteins, and carbonic acid. Let there be present in a solution approximately that amount of base which is ordinarily distributed between carbonic acid and serum proteins in normal blood, say 30 millimoles per liter, let the serum proteins amount to 50 grams per liter, and let the pressure of carbon dioxide be variable. The temperature is 37°. Then from the facts above discussed we may deduce the values of table 2. The two last rows of table 2 include the data necessary for the construction of a carbon dioxide dissociation curve of the system.

TABLE 2.

pH	6.61	6.83	7.12	7.42	7.61	7.83	8.12
[BP]	6.1	6.9	7.9	8.9	9.6	10.25	10.7
$[BHCO_3]$	23.9	23.1	22.1	21.1	20.4	19.75	19.3
$R=\frac{[H_2CO_3]}{[BHCO_3]}$ ..	1:3	1:5	1:10	1:20	1:30	1:50	1:100
$[H_2CO_3]$	7.97	4.62	2.21	1.05	0.68	0.395	0.193
Total CO_2	31.9	27.7	24.3	22.2	21.1	20.2	19.5

Next we may take the case of hemoglobin, carbonic acid, and base. We now choose the concentration of total base as 43 millimoles per liter, a fair value for the sum of the base distributed between hemoglobin and carbonic acid in normal arterial blood, the concentration of hemoglobin as 147 grams per liter. The other conditions are unmodified. Calculation gives table 3. It will be seen that table 3 must yield a dissociation curve of quite a different character from that of table 2.

TABLE 3.

pH	7.12	7.24	7.42	7.61	7.83	7.95
[BP]	7.8	13.0	20.5	28.2	36.2	42.6
$[BHCO_3]$	35.2	30.0	22.5	14.8	6.8	0.4
$R=\frac{[H_2CO_3]}{[BHCO_3]}$	1:10	3:40	1:20	1:30	1:50	1:67
$[H_2CO_3]$	3.52	2.24	1.125	0.493	0.136	0.006
Total CO_2	38.7	32.2	23.6	15.3	6.9	0.4

Finally we may take the case of all the proteins of normal blood plus carbonic acid and base. We now select 53 millimoles of base, 50 grams of serum protein, and 147 grams of oxyhemoglobin as the concentrations per liter. The system corresponds to blood, except that the solution is homogeneous. The results of the ensuing calculation are given by table 4.

TABLE 4.

pH	6.61	6.83	7.12	7.42	7.61	7.83	7.90
[BP]	6.5	14.1	24.5	35.2	41.7	49.4	52.0
$[BHCO_3]$	46.5	38.9	28.5	17.8	11.3	3.6	1.0
$R=\frac{[H_2CO_3]}{[BHCO_3]}$.	1:3	1:5	1:10	1:20	1:30	1:50	1:59
$[H_2CO_3]$	15.5	7.78	2.85	0.89	0.377	0.072	0.017
Total CO_2	62.0	46.7	31.4	18.7	11.7	3.7	1.0

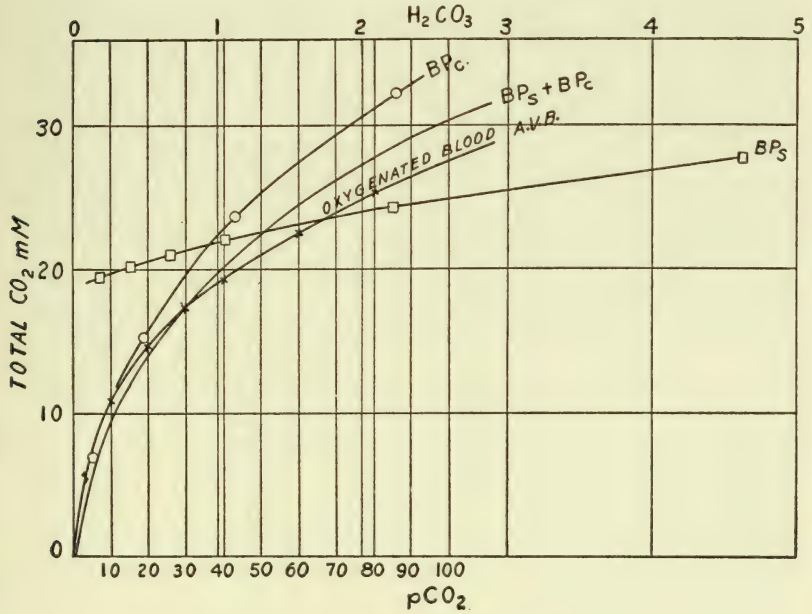


Fig. 9

Carbon Dioxide Dissociation Curves of Constituents of Blood

The carbonic acid dissociation curves obtained from these three tables are represented on figure 9, together with the curve of the blood of A.V.B. The similarity between the curve for normal blood and that for a solution of hemoglobin plus serum proteins is plain. The curve of the hemoglobin solution is also similar to that of blood, the curve of the solution of serum proteins much less so. But this curve closely resembles that of normal blood serum.

These simple instances are sufficient to show that the acid-base equilibrium of the proteins of blood, especially that of hemoglobin, determines the character of the carbon dioxide dissociation curves, at least for the case of normal blood, and makes possible the transport of carbonic acid.

The carbon dioxide dissociation curve of blood is, however, subject to variation. In a normal man it may be considerably lower during and after heavy work. Pathological changes are still greater. Thus in severe acidosis the curve is greatly lowered, in pernicious anemia slightly raised but greatly flattened, in certain forms of nephritis both lowered and flattened. There are also marked differences between different species of animals. For pathological conditions a large amount of information on this point may be found in a paper by Peters.[58] My collaborators at the Massachusetts General Hospital have also accumulated many measurements which are in course of publication.[59] All such changes may be imitated very closely by varying the amounts of base and of oxyhemoglobin in a solution like that described by table 3. For example, a small diminution in the amount of base will give a curve resembling that of the state of hard work, while a greater diminution leads to conditions resembling severe acidosis. Diminution of oxyhemoglobin leads to conditions resembling those observed in the blood of pernicious anemia,

[58] Peters, Eisenman, and Bulger, *Journal of Biological Chemistry,* LV, 709 (1923).

[59] *Journal of Biological Chemistry,* 1924 and following years.

diminution of both base and oxyhemoglobin to conditions similar to those of nephritis. In all such changes the serum proteins are of secondary importance, on account of their relatively low buffer value and relatively low concentration, but if the concentration of serum protein also be allowed to vary, somewhat closer approximations to the different blood curves may be obtained.

In these cases the artificial solutions not only yield curves which closely resemble those of blood, but also contain, in the same concentrations, the same substances that are present in blood. Thus proof is complete that the system above described gives, to a rough approximation, a true model of the changes in blood which are represented by the carbon dioxide dissociation curves. Nevertheless, small but unmistakable differences always subsist between the dissociation curves of such artificial systems and those of blood. These differences may be due in part to variations in activity coefficients and to the presence of neglected buffer substances such as phosphates, but they are chiefly the result of the existence in blood of two phases, cells and plasma. Under conditions to be found in the organism none of the substances which take part in the acid-base equilibrium, except free carbonic acid, occurs in even approximately the same concentration in the two phases, while in the case of the proteins there is not only a great difference in concentration, but also, as we have just seen, in chemical properties, between the constituents of the two phases.

Before undertaking a quantitative and theoretical consideration of this heterogeneous equilibrium, it will be convenient to examine the general characteristics of both the carbon dioxide and the oxygen dissociation curves of blood, a topic which has long engaged the attention of physiologists. The carbon dioxide dissociation curve of the oxygenated blood of a normal man at rest[60] is given

[60] Bock, Field, and Adair, *Journal of Biological Chemistry,* LIX, 371 (1924).

on figure 10. This is a smooth curve, approximately logarithmic in form. It will be seen that there is no upper limit to the increase of the total carbonic acid of blood with increase in pressure of carbon dioxide. This property of the curve is due to two facts. First, in accordance with Henry's law, the free carbonic acid dissolved in blood in-

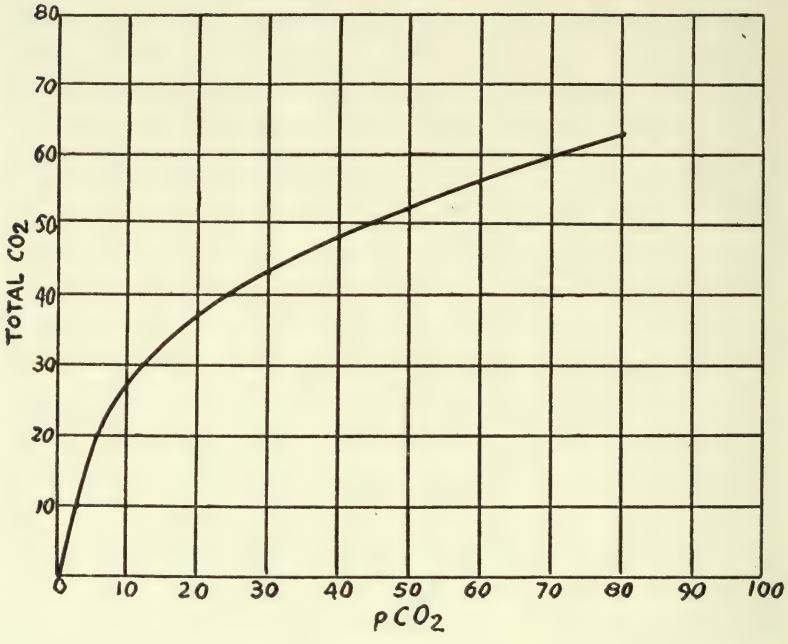


Fig. 10

Carbon Dioxide Dissociation Curve of Oxygenated Blood

creases in due proportion as gas pressure increases. Secondly, there is practically no limit, in normal blood, to the chemical reaction by which bicarbonate is formed as pressure increases, since the hydrogen ion concentration never rises high enough to exhaust the buffer action of the blood proteins.

When the partial pressure of carbon dioxide falls to 0, the blood loses all its carbonic acid, free and combined.

This is always true of normal human blood, because there is always more than enough protein to take up all the base bound by carbonic acid. For separated serum, however, this is not the case. In sum, the carbon dioxide dissociation curve of blood expresses the net result of all the processes which have just been analyzed.

Oxygen is absorbed by blood in small amounts as free dissolved oxygen and otherwise exclusively as the compound oxyhemoglobin, formed by chemical reaction between hemoglobin and oxygen. This reaction has been the subject of many investigations, but is not yet well understood either chemically or physico-chemically. For a pure dilute solution of hemoglobin the oxygen dissociation curve was formerly believed to bear some resemblance to a curve defined by the equation,

$$k = \frac{[\mathrm{Hb}]}{[\mathrm{HbO_2}]}\, \mathrm{pO_2},$$

which expresses the condition for equilibrium in the chemical reaction,

$$\mathrm{HbO_2} \rightleftharpoons \mathrm{Hb} + \mathrm{O_2}.$$

However, it is difficult to find hemoglobin solutions which fit this condition over a wide range of oxygen pressure, and experimental work with pure hemoglobin is very troublesome on account of the formation of methemoglobin and for other reasons. Also the shape of the curve is greatly modified, as Barcroft's numerous experiments have shown,[61] when the concentrations of electrolytes in the solution are varied. Finally, as we have already seen, there is evidence that the true reaction between oxygen and hemoglobin is

$$\mathrm{Hb_4O_8} = \mathrm{Hb_4} + 4\mathrm{O_2},$$

corresponding to a molecular weight of 67,000 for hemo-

[61] J. Barcroft, *The Respiratory Function of the Blood,* 1st ed., chap. IV, Cambridge, England, 1914.

globin. Such complications arising in the study of this reaction are far from surprising, since there is reason to expect the true thermodynamic concentrations of the substances concerned to vary with the conditions in a manner which escapes our present theoretical interpretations. We do know, moreover, that even in well-buffered hemoglobin solutions there occurs a change in hydrogen ion concentration accompanying change in oxygenation and this alone must be enough to produce a modification of the oxygen dissociation curves.

The real physiological phenomenon is, moreover, a less simple physico-chemical process, and we may well, for the present purpose, confine ourselves to the facts. It is a source of regret that the chemical reaction between oxygen and hemoglobin cannot be more satisfactorily described, but we shall find that lack of success at this point causes little inconvenience in the study of physiological problems.

In blood the conditions are approximately represented by Hill's equation,

$$k = \frac{[\mathrm{Hb}]}{[\mathrm{HbO_2}]} \cdot \mathrm{pO_2}^n.$$

Here the value of n, always much larger than one, is a variable with a characteristic value for each specimen of blood. But even this relation is not entirely satisfactory, for if values of the logarithm of the quantity $\frac{[\mathrm{Hb}]}{[\mathrm{HbO_2}]}$ be plotted against values of log $\mathrm{pO_2}$ the result is not a straight line, as it should be if the approximation were sufficiently precise. It is, however, possible to express the conditions accurately by graphical methods, and, in order to begin a discussion of the facts, we may consider four curves which present the results of Green and Ferry[62] and of Bock, Field and Adair.[63]

[62] Unpublished work.

[63] Bock, Field, and Adair, *Journal of Biological Chemistry,* LIX, 353 (1924).

Unlike the acid-base equilibrium in its simple features, which we have seen to be common to all natural waters, and unlike conditions of carbonic acid transport, which in general characteristics we have seen to be common to all protoplasm, the conditions defined by these curves consti-

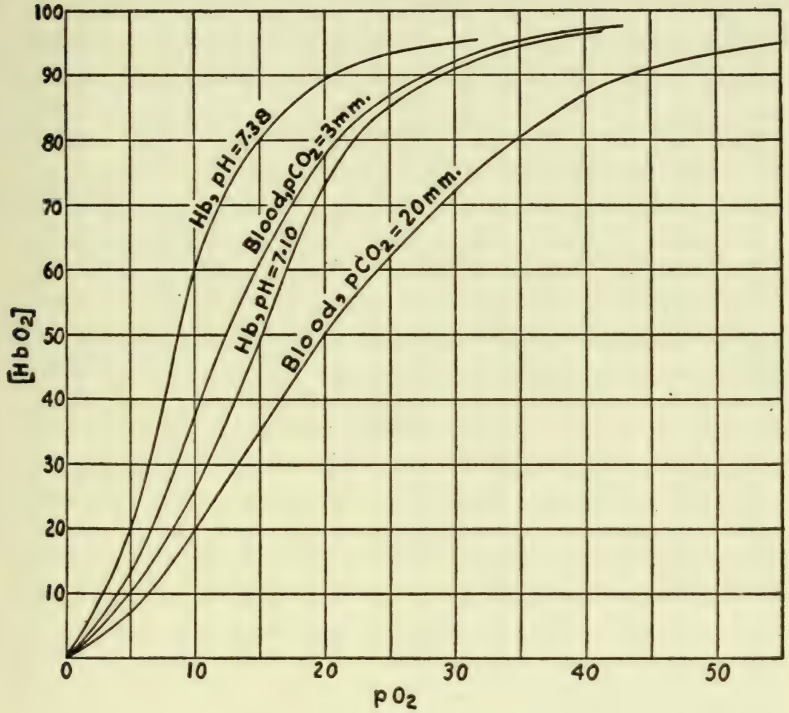


Fig. 11

Oxygen Dissociation Curves

tute a special functional adaptation. These conditions depend upon the properties of hemoglobin as they manifest themselves in the blood, and these properties alone make possible the transport of large quantities of oxygen from lungs to tissues. A carbon dioxide dissociation curve similar to that of blood, though representing a less efficient

transport of carbonic acid, is no less inevitable than a well-buffered hydrogen ion concentration near the neutral point. Both the curve and the buffering depend upon properties of substances common to all protoplasm and, with rare exceptions, to all liquids of the organism. On the contrary, the oxygen dissociation curve expresses the properties of the specialized substance, hemoglobin.

There are many contrasts between the state of oxygen and the state of carbonic acid in blood. Oxygen is bound by a single substance present only in the cells, but carbonic acid combines as bicarbonate with the base of both cells and plasma. The difference between the oxygen content of arterial blood and that of venous blood is great compared with the total amount of oxygen present in the blood, but for carbonic acid the corresponding difference is relatively small. The capacity of blood to combine with oxygen is limited by its hemoglobin content, and arterial blood is in fact nearly saturated with oxygen. But the capacity of blood to combine with carbonic acid has no definite limit and, as the pressure of carbon dioxide rises, combined carbonic acid may, therefore, increase almost indefinitely. Thus, while blood may be easily saturated with oxygen, the concept of blood saturated with carbonic acid is meaningless. As we have seen, carbonic acid, both free and combined, possesses other important properties in addition to its respiratory function. Oxygen, however, which is present in the plasma only in low concentration in solution in the free state is but indirectly involved in the other physiological functions of blood.

On account of greater solubility, the concentration of free carbonic acid in blood is very much greater than the concentration of free oxygen. The concentration of combined carbonic acid is also greater than the concentration of combined oxygen. In spite of these differences the absorption curves of the two substances are not altogether dissimilar. The oxygen curve, however, becomes almost horizontal in its upper ranges, while the carbon dioxide curve approaches very slowly an inclined straight line.

Also, the lower portion of the oxygen curve is S-shaped while the carbon dioxide curve is everywhere convex upward.

The forms of these curves aid in understanding the physiological functions of the blood. In the case of oxygen it is the upper nearly horizontal portion of the curve which is important in the lung. Evidently, even though alveolar oxygen pressure may vary widely under different circumstances, nearly complete saturation of the blood with oxygen is assured. A lower portion of the curve, approaching the vertical, represents conditions which exist in the tissues, especially where oxygen requirements are high. Under these circumstances the head of oxygen pressure may remain appreciable until almost all the combined oxygen has been used up. When the oxygen content of blood falls very low, the S-shape of the curve must be important. In the case of carbonic acid the conditions for arterial and venous blood are but slightly different. Yet the same differences in the slope of the curve in reverse order do exist.

It is convenient as a means of summing up all these facts to represent the two curves on the same figure and on the same scale. This has been done in figure 12. The curves (straight lines) for free oxygen and free carbonic acid are also given. Small rectangles have been added to enclose the fields of variation of oxygen and carbon dioxide pressures for the blood of A.V.B. at rest. It is easy to see from figure 12 that the convention according to which different units are employed in the representation of the two absorption curves has obscured important physiological relations.

The altitudes of the two rectangles of figure 12 show that for these curves the difference in absorbed carbonic acid corresponding to the ordinary difference in carbon dioxide pressure between arterial and venous blood is only about one-half the corresponding difference in the case of oxygen. But this is manifestly impossible, for it implies a respiratory quotient of only 0.5, instead of the

correct value, 0.8. This paradox, like others depending upon the same fact, has long been more or less apparent

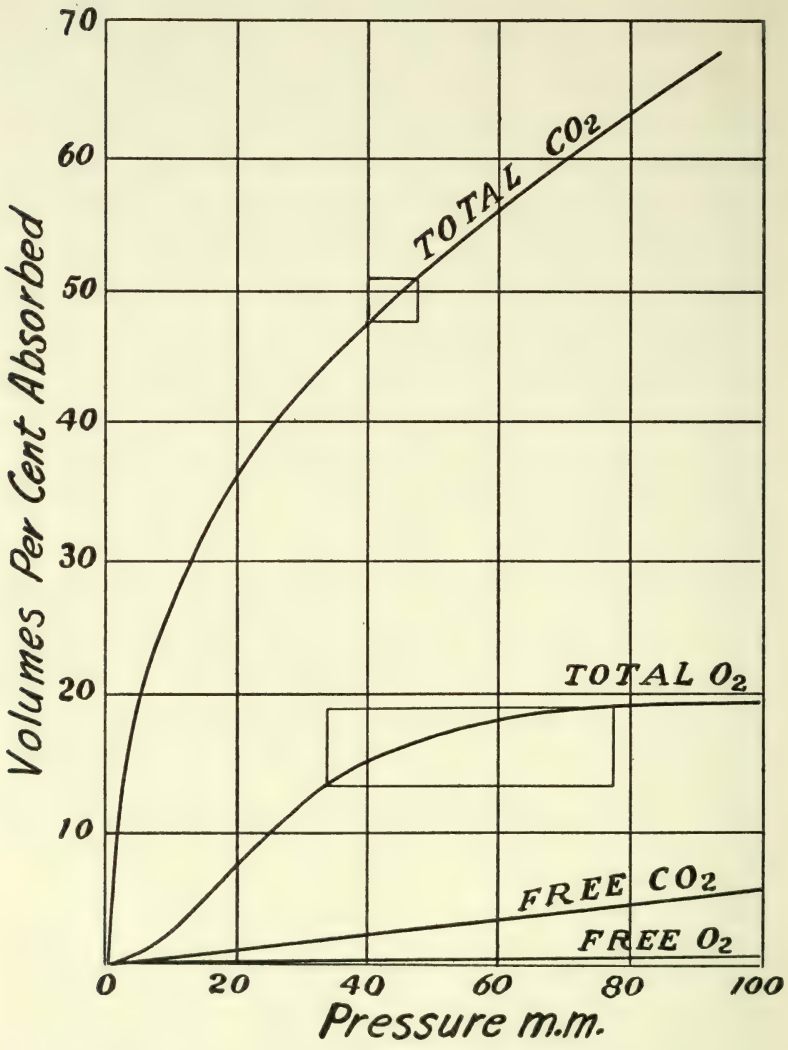


Fig. 12

Carbon Dioxide and Oxygen Dissociation Curves

and might have pointed the way to progress. But its meaning was not clearly grasped until experiment had brought forth new developments.

From the standpoint of the physiologist the conclusions which we have reached in this discussion of the absorption curves are but half truths because, in addition to all the other phenomena, an interaction between oxygen and carbonic acid takes place in blood. Long ago it was proved by Bohr, Hasselbalch, and Krogh that increase in concentration of free carbonic acid is accompanied by dissociation of oxyhemoglobin. The process was later carefully studied by Barcroft who confirmed the earlier observations and defined the equilibrium under widely varying conditions. The phenomenon is best illustrated by means of oxygen dissociation curves of the same blood at several different pressures of carbon dioxide.

On figure 13 four oxygen dissociation curves of the blood of A.V.B. are drawn. Reading from left to right, they correspond to partial pressures of carbon dioxide of

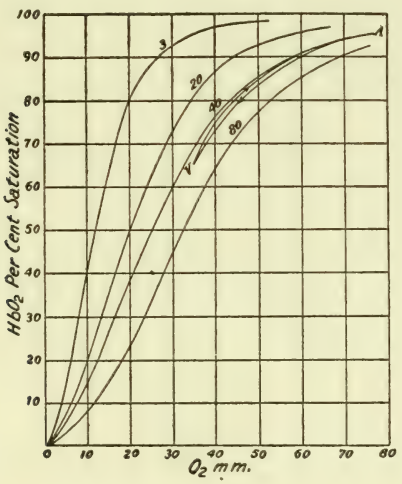


Fig. 13

Oxygen Dissociation Curves: Effect of Carbon Dioxide

3, 20, 40, and 80 mm. respectively. These curves are all similar, and all roughly approximate to curves of Hill's equation,

$$k = \frac{[\mathrm{Hb}]}{[\mathrm{HbO_2}]} \cdot \mathrm{pO_2}^n.$$

The values of k are, however, different from curve to curve and k is approximately a linear function of carbon dioxide pressure:

$$k = a \cdot \mathrm{pCO_2} + b.$$

Therefore the facts represented by the family of oxygen dissociation curves may also be represented by an equation of the form,

$$a \cdot \mathrm{pCO_2} + b = \frac{[\mathrm{Hb}]}{[\mathrm{HbO_2}]} \cdot \mathrm{pO_2}^n.$$

This is, indeed, a rough approximation. But it will serve, since it includes all the relevant variables for the case of a single specimen of blood. Needless to say, it would be possible to fit the curves with an equation as closely as experimental precision permits, but this is unnecessary for the present consideration.

At this point two conclusions may be deduced. First, the capacity for oxygen of a given sample of blood at a given pressure of oxygen is constant only when the pressure of carbon dioxide is also constant. Secondly, the fall in concentration of carbon dioxide in the lung is accompanied by a rise in the capacity of the blood to absorb oxygen, and the rise in concentration of carbonic acid in the tissues is accompanied by a fall in the capacity to absorb oxygen. Therefore the physiological process of oxygen transport is not represented by a single member of the family of oxygen dissociation curves of figure 13, but by a different curve, or rather cycle, which cuts across these curves. The cycle is shown on figure 13.

To those who have not themselves experienced that state of bewilderment which is the usual condition of the

investigator, it must seem strange that the physiologists who were studying the respiratory function of the blood should not have drawn from the discovery of the variation of oxygen saturation with carbon dioxide pressure the conclusion that, since carbonic acid influences the oxygen equilibrium in blood, oxygen must influence the carbonic acid equilibrium. If proof of so obvious a proposition is necessary a mere glance at the above equation will suffice, for if pO_2 and $\frac{[Hb]}{[HbO_2]}$ are functions of pCO_2, then pCO_2 is a function of pO_2 and of $\frac{[Hb]}{[HbO_2]}$. Yet, so little are physiologists accustomed to mathematics, and such is the natural inertia of the mind, that this conclusion escaped us all and it remained for Christiansen, Douglas, and Haldane[64] to discover by experiment that the carbon dioxide dissociation curves of oxygenated and of reduced bloods are different. The facts for the blood of A.V.B. are given by the two curves of figure 14.

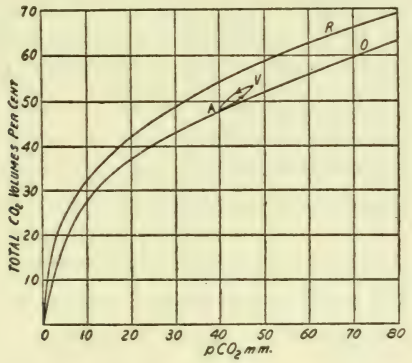


Fig. 14

Carbon Dioxide Dissociation Curves: Effect of Oxygen

[64] Christiansen, Douglas, and Haldane, *Journal of Physiology,* XLVIII, 244 (1914).

It is now known that when blood is partially oxygenated its carbon dioxide dissociation curves fall between these two extremes at levels proportional to the degree of oxygenation. All these curves are approximately logarithmic. Clearly the physiological exchanges in the case of carbonic acid, as in the case of oxygen, are not represented by one of this family of curves, but by a cycle which cuts across them. This cycle is drawn on figure 14.

When the cycles of figures 13 and 14 are taken into account, the discrepancy between the physiological facts, which is manifested by the anomalous apparent value of 0.5 for the respiratory quotient, disappears. That is due to a much greater effect of oxygenation and reduction of hemoglobin upon the carbonic acid cycle than of changing carbonic acid concentration upon the oxygen cycle. It is roughly indicated by the fact that the carbonic acid cycle is more divergent from the dissociation curves than is the oxygen cycle. We shall return more than once to a quantitative discussion of this question.

In studying the interaction between oxygen and carbonic acid, it is of the first importance not to regard the change in one substance as cause and the change in the other as effect. If we think of our terms mathematically as variables and functions, the difficulty does not arise. This error is an example of one of the most familiar and one of the most natural of fallacies and it was responsible for the long delay in reaching an understanding of carbonic acid transport. What it is important to bear in mind is that there exists a reciprocal relation between the two substances, oxygen and carbonic acid. In the lung the escape of carbonic acid facilitates the absorption of oxygen and the absorption of oxygen facilitates the escape of carbonic acid. In the tissues the inverse relations hold and in a like manner facilitate the exchanges of the two substances. Nevertheless, as above suggested, there is a great difference between the magnitudes of the variations in oxygen and carbonic acid concentrations which are thus

determined. Under ordinary conditions in the lung this disparity is in the ratio of about one to ten.

The theoretical interpretation of these interactions involves a return to the study of the acid-base equilibrium. When the differences between the oxygenated and reduced carbon dioxide dissociation curves had been experimentally determined, Haldane[65] perceived that the facts are consistent with the hypothesis that oxygenation makes the blood more acid. The theory of the process was later discussed by Parsons[66] and by me.[67] We shall here follow the last of these discussions. Given the data necessary for the construction of a carbon dioxide dissociation curve, it is possible to calculate the approximate values of the hydrogen ion concentration in blood. Strictly speaking, this concentration is a meaningless concept, for there is no such thing as the hydrogen ion concentration of blood as a whole, but only the hydrogen ion concentration in cells and plasma separately. Nevertheless, for small variations in the values of these variables such as those which occur along an ordinary carbon dioxide dissociation curve, whether oxygenated or reduced, the hydrogen ion concentrations of both cells and plasma are approximately linear functions of the ratio of carbonic acid concentration and bicarbonate concentration in whole blood. Thus it is possible to express the combined carbonic acid (bicarbonate) of blood as a function, not of carbon dioxide pressure, but of the hydrogen ion concentration. Haggard and Yandell Henderson[68] have made important use of this fact in a nomographical extension of the ordinary dissociation curves.

In the form of two curves figure 15 gives the results of the calculation of bicarbonate concentration as a function

[65] Christiansen, Douglas, and Haldane, *Journal of Physiology,* XLVIII, 244 (1914).

[66] T. R. Parsons, *Journal of Physiology,* LIII, 42 (1919-1920).

[67] L. J. Henderson, *Journal of Biological Chemistry,* XLI, 401 (1920).

[68] Haggard and Y. Henderson, *Journal of Biological Chemistry,* XXXIX, 163 (1919).

of hydrogen ion concentration for both reduced and oxygenated bloods. The curves are similar to the dissociation curves from which they have been derived, but they permit an important deduction. Evidently the points on the two curves which fall on the same abscissa correspond to approximately the same hydrogen ion concentration of plasma or of cells. Now the curve for reduced blood is everywhere higher than the curve for oxygenated blood.

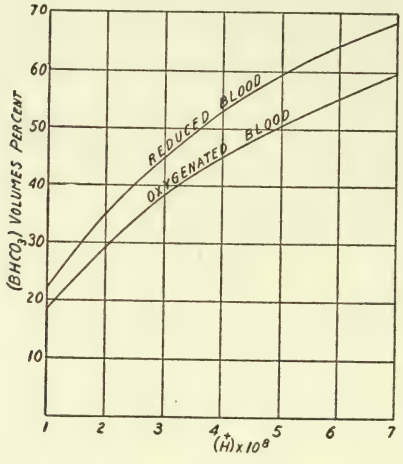


Fig. 15

Bicarbonate, Oxygen, and Hydrogen Ion Concentration

In other words, when hydrogen ion concentrations are equal reduced blood always combines with more carbonic acid than does oxygenated blood. How is this possible? The only chemical reaction directly involved in oxygenation is the formation of oxyhemoglobin. Therefore, oxyhemoglobin must be either a more acid or less alkaline substance than reduced hemoglobin. The mechanism of the process is still uncertain, but the fact is beyond doubt. The simplest hypothesis to explain the fact assumes, as

already suggested, that when oxygen combines with hemoglobin a single acid radical of hemoglobin in the neighborhood of the point of union of oxygen becomes stronger and, therefore, more highly ionized ($k'_O > k'_R$). On this assumption all facts can be quantitatively accounted for. Evidently, increase of acidity of this radical must lead to decomposition of bicarbonate, decrease of acidity to a formation of bicarbonate in the solution. Moreover, if combination of hemoglobin with oxygen leads to a change in the affinity of this acid radical, then change in the ionization of the acid radical must lead to change in affinity for oxygen. By application of this hypothesis the cycle of changes may be interpreted.

My original estimates of the values of k' for this acid radical were very rough approximations because the above treatment is inadequate for accurate quantitative purposes, and because it had not been discovered how different are conditions in the cells from those in the plasma and how exceptionally large is the amount of base with which hemoglobin combines. Recently the question has been carefully studied in Van Slyke's[69] laboratory. The results may be summed up in the statement that known facts are consistent with the theory that the radical of hemoglobin in question behaves in the manner above suggested and that it is characterized by the following values of k' in reduced hemoglobin and oxyhemoglobin respectively:

$$k'_R = 6.6 \times 10^{-9},$$
$$k'_O = 2.4 \times 10^{-7}.$$

Experimental observations and theoretical relations are well presented in figure 16 taken from Van Slyke's valuable monograph.[70]

[69] Hastings, Van Slyke, Neill, Heidelberger, and Harington, *Journal of Biological Chemistry,* LX, 89 (1924).

[70] D. D. Van Slyke, *Factors Affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body,* Philadelphia and London, 1926.

It has long been apparent to those who have subjected the facts to a critical scrutiny that simple buffer action of the proteins of blood must be insufficient to account for the amount of base taken up by carbonic acid in the tissues and liberated in the lung. This conclusion arose from my early quantitative studies of buffer action.[71]

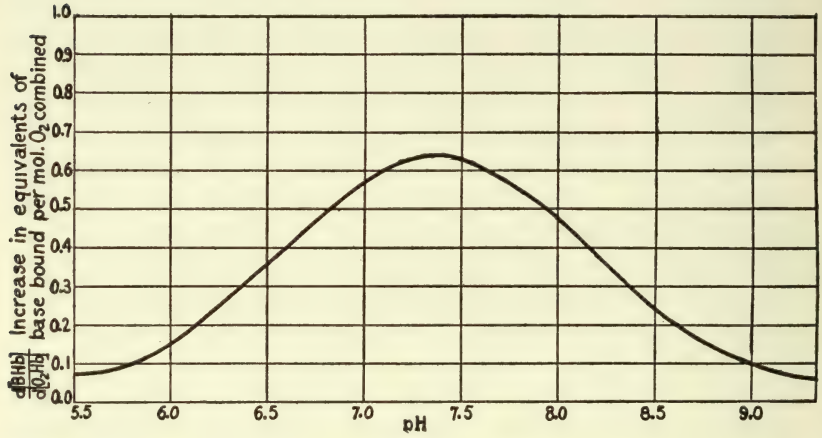


Fig. 16

Theory of the Oxygen Effect

Taking the best modern values, in order to obtain consistency with later discussions, we may make the following estimates of what conditions would be in the respiratory exchange of carbonic acid in the blood but for the heterogeneity of the system and the difference in state of the hemoglobin molecule in arterial and venous blood. These estimates are in the form of the differences in composition between arterial and venous blood which would arise if it were possible to suppress the effects of hetero-

[71] L. J. Henderson, *Ergebnisse der Physiologie,* Jahrgang VIII, p. 254 (1909).

geneity and of changing acidity of the hemoglobin molecule:

$$\Delta[BHCO_3] = 2.08 \text{ millimoles per liter},$$
$$\Delta pH = 0.028,$$
$$\Delta[BP]_c = 0.88 \text{ millimole per liter},$$
$$\Delta[BP]_s = 0.09 \text{ millimole per liter},$$
$$\Delta[BP]_B = 0.97 \text{ millimole per liter}.$$

These values indicate that the true buffer action of all the blood proteins, $\Delta[BP]_B$, is less than half that of carbonic acid, $\Delta[BHCO_3]$. A similar consideration based upon less accurate data, and, like this, only roughly approximate, because of ignorance of the acid-base relations within the cell and of the laws of heterogeneous equilibrium, led me to the conclusion that an unknown factor within the cells supplements simple buffer action and facilitates the transport of carbonic acid.[72] This unknown factor is the effect of oxygenation upon the acid properties of hemoglobin.

The problem may be clarified by a consideration of the composition of the blood of A.V.B. under several different conditions as defined in table 5.

In this table, column I gives the composition of arterial blood, column II that of venous blood, column III that of blood having the oxygen content of arterial blood but the same value of pCO_2 as venous blood, while column IV gives the value of BP_c for blood having the same value of HbO_2 as arterial blood and the same value of pH_c as venous blood.

[72] L. J. Henderson, *Journal of Biological Chemistry*, VII, 29 (1909).

TABLE 5.

	I Arterial blood	II Venous blood	III	IV	II − I	III − I	IV − I
(HbO_2)	96	65	96	96			
pCO_2	40	47	47				
$(BHCO_3)_B$	20.44	22.52	21.67		+2.08	+1.23	
pH_s	7.450	7.421	7.405		−0.029	−0.045	
pH_c	7.309	7.300	7.274	7.300	−0.009	−0.035	
BP_s	9.20	9.09	9.04		−0.11	−0.16	
BP_c	22.70	20.73	21.63	22.44	−1.97	−1.07	−0.26
BP_B	31.90	29.82	30.67		−2.08	−1.23	

It will be seen that simultaneous changes in oxygenation and in partial pressure of carbon dioxide, such as actually take place in the respiratory cycle, involve an increase of 70 per cent in the variations of $(BHCO_3)_B$ and BP_B compared with the case when, oxygenation remaining constant, there is an equal change in pCO_2. But, in the former case, the change in pH_s is only two-thirds that in the second case, the change in pH_c only one-fourth. Moreover, comparing columns I and IV, we see that change in hydrogen ion concentration of the cells equal to that which takes place in the respiratory cycle, when unaccompanied by change in oxygenation, produces a variation in BP_c of but 0.26 millimole per liter of blood, which is only about one-eighth of the variation which occurs during the respiratory cycle.

From these facts a number of conclusions may be drawn. First, in the normal respiratory cycle the true buffer action of hemoglobin is but $\frac{26}{197}$ or 13 per cent of the change in BP_c, hence 87 per cent of this change, or 1.71 millimoles per liter, is due to the change in oxygenation. Secondly, the total buffer action of the proteins is but $\frac{37}{208}$ or 18 per cent of the whole change in BP_B, the oxygenation of hemoglobin being responsible for the rest or 82 per cent of this quantity, and, therefore, for 82 per cent of the carbonic acid absorbed in the tissues and released in the lungs. Thirdly, hemoglobin, as a result of oxygenation plus buffer action, is responsible for $\frac{197}{208}$ or 95 per cent of the absorption and excretion of carbonic acid. Finally, the serum proteins are responsible for but $\frac{11}{208}$ or five per cent of this quantity. The oxygen effect is very large, far larger indeed than could have been foreseen when the decrease in variation of hydrogen ion concentration which accompanies it was unknown.

It will be unnecessary to continue this analysis of the absorption of oxygen and carbon dioxide by blood. From the point that we have now reached substantial progress is hardly possible except by means of a synthetic treatment of the blood as a physico-chemical system. But before this treatment can be undertaken, it remains to analyze one more class of facts. These relate to the heterogeneous equilibrium between cells and plasma.

CHAPTER V

CELLS AND PLASMA

THE unequal concentration of substances common to the two phases of blood and in part capable of passing from one to the other is the subject of the present chapter. We shall find it convenient to regard the conditions as arising from the presence of unequal quantities of protein in the two phases, from the unequal affinities of hemoglobin and serum proteins for base, and from the effects of oxygenation on this affinity in the case of hemoglobin.

In normal human blood plasma the concentration of base bound by protein is approximately one-fifth the concentration of the similar compound within the red cells. Therefore the protein salts of the plasma contribute far less to the osmotic pressure of their phase than do the protein salts of cells to that of the other phase of the system. Such an inequality of concentration must involve other inequalities if osmotic equilibrium is to exist; and this equilibrium does exist, uncomplicated by mechanical pressure.[73] Thus it may be seen that the distribution of dissolved substances between cells and plasma is a complex phenomena.

A clear understanding of the nature of the heterogeneous equilibrium in blood has been but recently attained, long after most of the other facts which we have studied had been satisfactorily interpreted. Yet the first observation on this subject is now sixty years old and precedes nearly everything thus far considered in this book. In

[73] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 765 (1923).

1867 and 1868 Zuntz[74] proved that in normal blood the cells contain only about one-half as much carbonic acid (bicarbonate) as the serum. Yet, after saturating the blood with carbon dioxide at atmospheric pressure, he observed an additional formation of bicarbonate three times as great as that which occurred in serum that had been separated from cells. From this fact he concluded that the alkali which is available for the formation of bicarbonate in blood is chiefly contained in the cells. But he also observed that the additional quantity of bicarbonate thus formed in the presence of cells is not found in them, but occurs in large part in the plasma. Therefore, in addition to free carbonic acid some other acid or base had moved between cells and plasma. Similar observations were also made by A. Schmidt.[74a]

Twenty-five years later von Limbeck[75] made a second observation. Upon measuring the volume of the cells under different conditions he found that this volume increases with increase in the partial pressure of carbon dioxide. Such a change can be the result of but one process, a movement of water from serum to cells. This fact had, indeed, been discovered much earlier by H. Nasse,[75a] but the discovery passed unnoticed.

A crude imitation of both these phenomena was discovered by Spiro and me.[76] We constructed artificial cells with collodion membranes, filled them with a solution of serum globulin plus sodium bicarbonate and placed the cells in a similar solution of pure sodium bicarbonate. After equilibrium had been more or less precisely established, carbon dioxide was passed simultaneously through

[74] N. Zuntz, *Centralblatt Med. Wiss.*, 529, 1867; *Beiträge zur Physiologie des Blutes, Dissertation,* Bonn, 1868.

[74a] A Schmidt, *Ber. K. Säche. Ges. Wiss.,* I, xix, 30 (1867).

[75] R. von Limbeck, *Archiv für Experimentelle Pathologie und Pharmakologie,* XXXV, 309 (1894).

[75a] H. Nasse, *Pflüger's Archiv,* XVI, 604 (1878).

[76] Spiro and Henderson, *Biochemische Zeitschrift,* XV, 114 (1909).

both phases and we observed that under these circumstances base passed out of the artificial cell and water into it. Similar processes occurred when, for the protein, finely divided calcium carbonate was substituted. It was also possible to observe the phenomenon when discs of gelatine made up from a solution of sodium bicarbonate were substituted for the artificial cells.

In these simple experiments there can be no doubt of the nature of the process. At the outset a close approach to equilibrium, though not equality of concentrations, existed between the phases. When the system was saturated with carbon dioxide the cell phase, which contained the buffer, took up large quantities of carbonic acid as bicarbonate and thereby increased its osmotic pressure greatly and its acidity slightly. Meanwhile, the unbuffered phase absorbed only free carbonic acid, thereby increasing its osmotic pressure slightly, but its acidity greatly. Therefore water passed into the cell on account of the disturbed osmotic equilibrium and base passed out, both because of the disturbed osmotic equilibrium and also because of the disturbed heterogeneous acid-base equilibrium.

This explanation applies, but as we were well aware applies imperfectly, to the blood. It had, in fact, already been discovered by Gürber[77] that while bicarbonate and chloride ions move in and out of the red cells, base does not. Therefore, though it was apparent that the movement of chloride ion in one direction is, for the acid-base equilibrium, equivalent to a movement of sodium in the other direction, much remained to be accounted for; how much we were then far from clearly understanding.

Already, however, a quantitative study of the hydrogen ion concentration changes in the blood, and of the buffer values of protein had led me to the conclusion that the transport of carbonic acid is certainly not entirely explicable as far as plasma is concerned and probably not

[77] A. Gürber, *Maly's Jahresbericht,* XXV, 164 (1895).

in whole blood by means of simple buffer action of the proteins and bicarbonates[78] through the reaction,

$$BP + H_2CO_3 \rightleftharpoons BHCO_3 + HP.$$

This subject has been partly explained in the preceding chapter. The facts discovered by Zuntz and Gürber, when interpreted by means of the theory that Spiro and I had stated seemed to overcome the difficulties concerning plasma, but the problem of the cells remained obscure. For, while it was not difficult to make out a good case for the probability that simple buffer action is inadequate to account for what was then known to occur, the theory afforded no opportunity of quantitatively characterizing the heterogeneous equilibrium. In fact three pieces of information were lacking; the quantitative description of the heterogeneous equilibrium, the titration curve of hemoglobin and, what was then unsuspected, the effect of oxygen upon this curve. Of these points we have already considered the second and third in preceding chapters.

The need of a solution of the first problem was obvious, and in 1910 I undertook the study of equilibrium in a two-phase system, in which one phase consisted of the solution of an acid, the other of the same solution plus a protein. But I lacked the wit to carry these experiments to a successful conclusion. This was partly due to the fact that the problem contains a concealed pitfall and that for this reason the case of the blood is in one respect actually simpler than the case of this seemingly simple system.

At least one other conclusion, however, was clear, as a result of the discovery of the exchange of chloride between cells and plasma. The apparent movement of base or of bicarbonate between the two phases must be the result of the actual movement of hydrochloric acid or, to state the facts in terms of a reasonable theory, of the exchange of chloride for bicarbonate ions across the cell wall. In its simplest form the process may be represented

[78] L. J. Henderson, *Journal of Biological Chemistry,* VII, 29 (1909).

as follows. Let the cells be conceived as a solution of hemoglobin functioning as an acid plus carbonic and hydrochloric acids in equilibrium with base, the plasma as a similar solution in which hemoglobin is replaced by the serum proteins. Then increase of concentration of free carbonic acid must lead to the formation in the cells of a larger quantity of bicarbonate through buffer action, at the expense of salts of hemoglobin, than is formed by a similar reaction in the plasma. In fact such action in the plasma, because much smaller, may be provisionally neglected. This process is accompanied by the above-mentioned exchange of some of the bicarbonate ions of the cell for an equal number of chloride ions of the plasma. The result is a large increase in plasma bicarbonate and an apparent transfer of base from cells to plasma.

In short, an increase of carbon dioxide partial pressure in blood leads to increase of the sum of $[BP] + [BHCO_3]$ in serum and to decrease of the sum of these quantities in cells (Zuntz), to increase of cell water and of cell volume and to decrease of plasma water and of plasma volume (Nasse), and to increase of cell chloride and to decrease of plasma chloride (Gürber). All these changes are the results of the movement of water and of chloride ions into the cells and of the movement of bicarbonate ions out of the cells. When the partial pressure of carbon dioxide falls the processes are reversed, water and chloride ions move out of the cells, bicarbonate ions into them, and corresponding changes in volume and in distribution of bicarbonate result. In other words, distribution of water and of chloride and bicarbonate ions must be mathematical functions of the partial pressure of carbon dioxide.

Long after the nature of these facts had become apparent, the discovery by Christiansen, Douglas, and Haldane[79] of the effect of oxygenation of blood upon its carbon dioxide dissociation curves finally led me to the conclusion which should have been drawn a decade earlier,

[79] Christiansen, Douglas, and Haldane, *Journal of Physiology,* XLVIII, 244 (1914).

that every one of the variables involved in the respiratory exchanges of blood must be a mathematical function of all the others. In particular, since the distribution of water, of bicarbonate and of chloride between cells and serum are functions of carbon dioxide pressure and carbon dioxide pressure is a function of oxygen pressure, the distribution of water, of bicarbonate, and of chloride must also be functions of oxygen pressure. Therefore, it should be possible to observe the phenomena of Zuntz, of Nasse, and of Gürber in blood when oxygen pressure is varied. Accordingly, I proposed to F. C. McLean who had already become interested in the physiological behavior of chlorides and who was about to begin work in my laboratory, that he should study the effect of oxygen pressure upon the concentration of chlorides in blood. McLean had no difficulty in verifying the deduction and in making approximate measurements of the variations in chloride content of serum under the influence of changing oxygen pressure in whole blood.[80] The facts are analogous to those discovered by Bohr, Hasselbalch, and Krogh[81] concerning the effects of oxygen and carbon dioxide pressure upon the absorption of oxygen by blood, and to those discovered by Christiansen, Douglas, and Haldane.[82] They may be illustrated by figure 17 which is a transformation of a portion of the figure in which our results were summed up. Here ordinates represent concentrations of chloride in serum, abscissas oxygen pressure, while each curve corresponds to one constant pressure of carbon dioxide. After comparison with figure 13, page 79 there will be no difficulty in understanding the facts.

At this stage it finally became possible to piece to-

[80] L. J. Henderson, *Journal of Biological Chemistry,* XLVI, 411 (1921).

[81] Bohr, Hasselbalch, and Krogh, *Skandinavisches Archiv für Physiologie,* XVI, 412 (1907).

[82] Christiansen, Douglas, and Haldane, *Journal of Physiology,* XLVIII, 244 (1914).

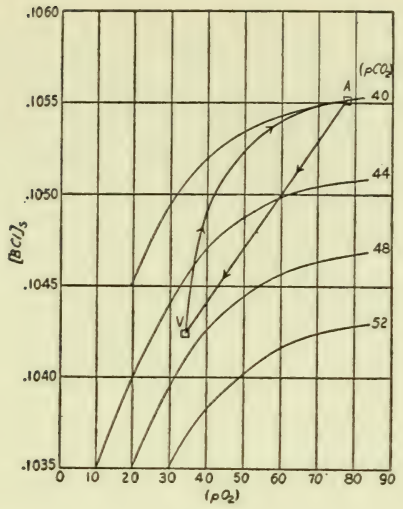


Fig. 17

Chloride Distribution and Oxygen Pressure

gether the various fragments of information which have engaged our attention in this and the preceding chapter, and to construct the Cartesian nomogram[83] here represented as figure 18. This is a graphical representation of all the facts which were quantitatively known eight years ago concerning the respiratory activities of ordinary mammalian blood. We shall return to this subject in the next chapter.

McLean's experiments had established the facts concerning the chloride equilibrium, but we feared that they were as yet hardly accurate enough, in view of the growing importance of the subject. Moreover, the problem of water balance between cells and plasma had been left untouched. It was also apparent that in due course theoretical interpretations of the phenomenon better proportioned to the increasing power of theoretical physicochemical analysis would be required. In view also of the

[83] L. J. Henderson, *Journal of Biological Chemistry,* XLVI, 411 (1921).

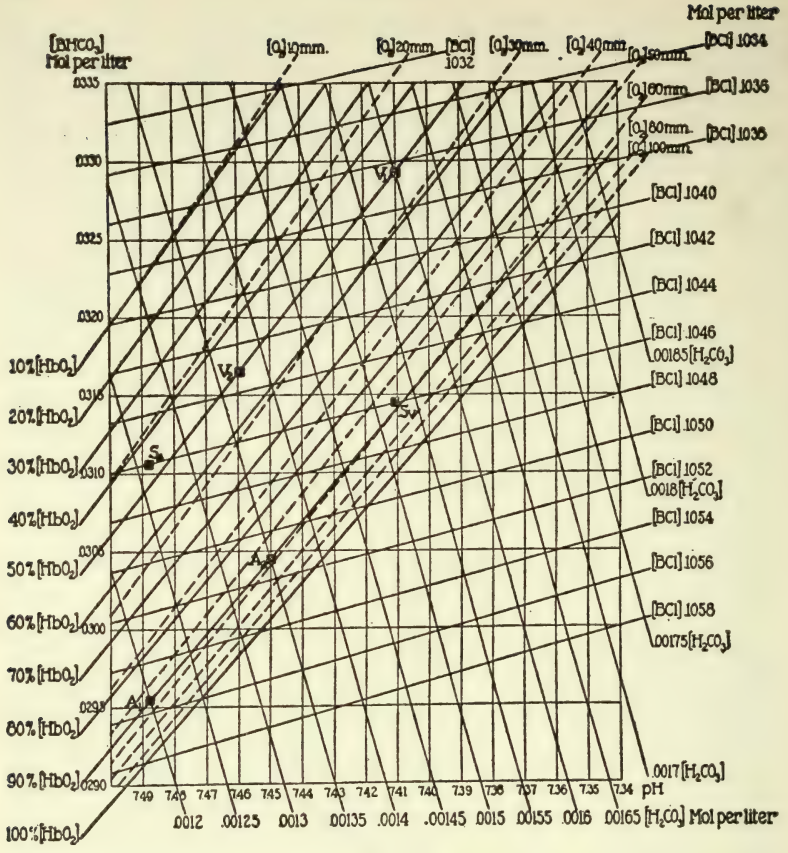


Fig. 18

Cartesian Nomogram for Mammalian Blood

complexity of the problem it was agreed that the work should be continued in Van Slyke's laboratory at the Rockefeller Institute as well as in my own, and that McLean working with Van Slyke should continue the study of the heterogeneous equilibrium. Thus began a collaboration which was later continued in Peking with the aid of Wu and which has resulted in what now appears to be the approximate solution of all the questions at issue. The results may be found in the important paper of Van Slyke,

Wu, and McLean,[84] which is equally admirable as a theoretical study and as an experimental research. Some of the aspects of this intricate problem had been previously treated in a valuable paper by Warburg.[84a] This paper, in which many of the theoretical refinements of the subject were first worked out, is very useful for its historical as well as for its theoretical discussions.

Theoretically, the first step in this investigation consisted in the treatment of the problem above referred to of equilibrium between a protein and an acid or base in one phase and acid or base alone in solution in the other phase of a two-phase system.

Meanwhile the problem in its general form had been solved by Donnan,[85] who, as the developments of recent years have shown, thereby made a contribution of the first importance to physiology. Like so many other independent discoveries of modern physical chemistry, Donnan's results are to be found in Gibbs's great memoir,[86] where they had lain unnoticed. The Gibbs-Donnan law of heterogeneous equilibrium applies to heterogeneous systems when, in at least one of the phases, there is a substance which cannot pass out of the phase in which it is present, but which does combine with another substance that is not itself thus restricted.

The proteins, together with the bases and acids with which they form salts, manifest such behavior and there is accordingly hardly a physiological process in which this law is not somewhere involved and in which it may not be invoked with profit.

The Gibbs-Donnan law, a deduction from elementary thermodynamical principles, may be formulated for present purposes as follows: Given a system of two phases,

[84] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 765 (1923).

[84a] E. J. Warburg, *Biochemical Journal,* XVI, 153 (1922).

[85] W. C. McC. Lewis, *A System of Physical Chemistry,* 3d ed., vol. II, London, 1920.

[86] J. Willard Gibbs, *Scientific Papers,* I, 83, London, 1906.

S and C, let there be present in phase C the substance P which is restricted to this phase and which is capable of forming ionized salts PA_1, PA_2, . . . with acids. In both phases there are present acids yielding ions A_1^-, A_2^-, . . . and bases yielding ions B_1^+, B_2^+. . . . The ions A_1^-, A_2^-, . . ., B_1^+, B_2^+, . . . exist in both phases and are capable of passing from one to the other. In both phases the solvent is water. Let the true thermodynamical concentrations or activities of the ions be (a_1), (a_2), . . . (b_1), (b_2), . . . and let their respective valences be p_1, p_2, . . ., q_1, q_2. . . . Then a necessary condition for equilibrium is as follows:

$$\frac{(a_1)_c^{1/p_1}}{(a_1)_s^{1/p_1}} = \frac{(a_2)_c^{1/p_2}}{(a_2)_s^{1/p_2}} = \ldots = \frac{(b_1)_s^{1/q_1}}{(b_1)_c^{1/q_1}} = \frac{(b_2)_s^{1/q_2}}{(b_2)_c^{1/q_2}} = \ldots = r. \quad (1)$$

If $p = q = 1$, which is the case for the ions Cl^-, HCO_3^-, OH^-, and H^+ with which we shall be concerned, we have:

$$\frac{(a_1)_c}{(a_1)_s} = \frac{(a_2)_c}{(a_2)_s} = \ldots = \frac{(b_1)_s}{(b_1)_c} = \frac{(b_2)_s}{(b_2)_c} = \ldots = r. \quad (2)$$

Equation 2 may be employed, as Van Slyke, Wu, and McLean have shown, in the present case.

We shall now consider the simplest possible model of the Gibbs-Donnan effect in blood as it has been worked out by Van Slyke, Wu, and McLean. Let there be present cells containing an ion B^+, which is in fact chiefly potassium, hemoglobin in an ionized condition, and an ion A^-, which may be taken to represent chiefly chloride and bicarbonate. In the serum let there be present the same ion A^- and an equivalent concentration of ion B^+, which is here chiefly sodium. Neither hemoglobin nor B^+ (*i.e.*, sodium and potassium) can pass from one phase to the other. The conditions in normal blood are approximately those indicated in figure 19 which calls for no explanation except that the area marked Hb^- represents the equivalent concentration of *ionized* hemoglobin. This area therefore measures the quantity of B^+ which is neutralized by hemoglobin under the conditions of equilibrium represented by this particular diagram. It must be clearly un-

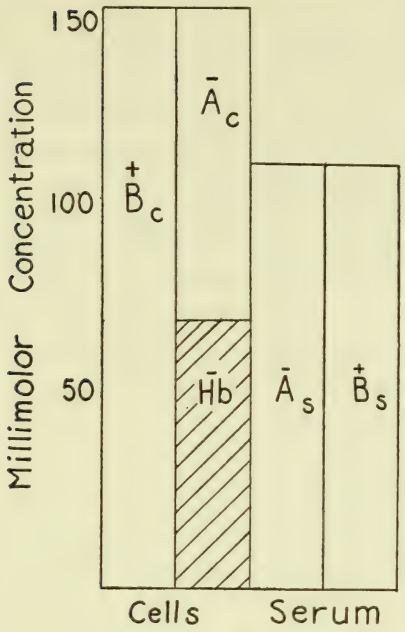


Fig. 19

Blood: The Heterogeneous Equilibrium. Approximate Description

derstood, as already explained, that while the *mass* of hemoglobin remains constant, this area will vary both with the hydrogen ion concentration of the cells, in accordance with the titration curve, and also with the state of oxygenation of the hemoglobin.

From the above discussion we have

$$\frac{(A^-)_c}{(A^-)_s} = \frac{(Cl^-)_c}{(Cl^-)_s} = \frac{(HCO_3^-)_c}{(HCO_3^-)_s} = \frac{(H^+)_s}{(H^+)_c} = r. \qquad (3)$$

Also, since there is electrical neutrality,

$$(B^+)_c = (A^-)_c + (Hb^-), \qquad (4)$$

and

$$(B^+)_s = (A^-)_s. \qquad (5)$$

Finally, since osmotic equilibrium exists,

$$(B^+)_c + (A^-)_c = (B^+)_s + (A^-)_s. \qquad (6)$$

Equation 6 neglects the osmotic effect of hemoglobin and all the equations (3, 4, 5, and 6) must be regarded as rough approximations. They neglect, in fact, serum proteins, divalent ions and, as they must be at present applied, activity coefficients. Figure 19 is a graphical representation of the conditions defined by these equations. Thus the area B_c^+ is equal to the sum of the areas $A_c^- + Hb^+$ (equation 4). The area B_s^+ is equal to the area A_s^- (equation 5), the sum of the areas $B_c^+ + A_c^-$ is equal to the sum of the areas $B_s^+ + A_s^-$ (equation 6), and finally the ratio of the area A_c to the area A_s is equal to r (equation 3). Addition of the term (Hb^-) to both sides of equation 6 gives the relation,

$$(B^+)_c + (A^-)_c + (Hb^-) = (B^+)_s + (A^-)_s + (Hb^-), \quad (7)$$

which shows that the total ionic concentration of the cells, represented by the left-hand side of equation 7, is greater than that of the serum by the value (Hb^-). Substitution in equation 7 of the values of $(B^+)_c$ and $(B^+)_s$ from equations 4 and 5 gives

$$2(A^-)_c + 2(Hb^-) = 2(A^-)_s + (Hb^-),$$

or

$$(A^-)_c + (Hb^-) - (A^-)_s = \frac{(Hb^-)}{2}. \quad (8)$$

In words the total concentration of anions in cells is greater than that in serum by a quantity equal to one-half the concentration of ionized hemoglobin. This is also true for the concentration of cations, while, on the other hand, the value of $(A^-)_c$ is less than that of $(A^-)_s$ by the same quantity:

$$(A^-)_c + \frac{(Hb^-)}{2} = (A^-)_s. \quad (9)$$

Therefore, with equation 3, we have

$$r = \frac{(H^+)_s}{(H^+)_c} = \frac{(A^-)_c}{(A^-)_s} = 1 - \frac{(Hb^-)}{2(A^-)_s}. \quad (10)$$

The quantity $1 - \frac{(Hb^-)}{2(A^-)_s}$ is accordingly, in a rough approximation, a measure of the value of r and therefore of the distribution of chloride and bicarbonate between cells and serum. As Van Slyke has pointed out: "The greater the concentration in the cells of Hb^-, equivalent to the base neutralized by hemoglobin, the farther will the value of r be depressed below 1, and hence the greater will be the inequality of electrolyte distribution between cells and serum."

We have seen that the amount of base bound by hemoglobin increases as the degree of oxygenation of hemoglobin increases and decreases as the pressure of carbon dioxide increases. Hence the movement of chloride into the cells when pressure of carbon dioxide increases, as discovered by Gürber, and the movement of chloride out of the cells when oxygen pressure increases, as discovered by McLean and myself. In other words, during the respiratory cycle the quantities $pO_2, \frac{1}{pCO_2}, \frac{1}{(Cl^-)_c}, \frac{1}{(HCO_3^-)}, \frac{1}{r}$ all vary with the quantity (Hb^-) of the above discussion.

These conclusions show that the theory of Spiro and myself concerning the adjustment of the heterogeneous acid-base equilibrium might have been misleading. As we have seen, this theory is in accord with the facts, in case the heterogeneous equilibrium is disturbed by varying partial pressure of carbon dioxide. It is no less in agreement with the phenomena observed when oxygen pressure varies. But an increasing oxygen pressure causes chloride to move out of the cells, in spite of the fact that the blood is meanwhile becoming more acid, as it is also in the case of increasing carbon dioxide pressure, when chloride moves into the cells. Nevertheless, while increasing pressure of carbon dioxide causes a great increase in $(HCO_3^-)_c$ and a small increase in $(HCO_3^-)_s$, hence an in-

crease in r and *relative* acidity of serum, increasing oxygen pressure causes a decrease in $(HCO_3^-)_c$ and no direct change in $(HCO_3^-)_s$, hence a decrease in r and *relative* acidity of cells. Therefore, increase of pressure of carbon dioxide is accompanied by a movement of chloride ions into the cells, increase of oxygen pressure by a movement of chloride ions out of the cells. In short, the older theory is consistent with the facts and with the more recent theory as quantitatively developed by Van Slyke[87] from the Gibbs-Donnan law. But the older theory cannot of course explain the quantitative relations because it was put forth at a time when the magnitude of the differences in concentrations between cells and serums was unknown and theoretically inexplicable. The older theory takes account of the known increases or decreases of unknown differences between concentrations in cells and plasma, the theory of Van Slyke, Wu, and McLean of known values and ratios of these concentrations.

We may now proceed to a more detailed consideration of the magnitudes of the changes involved in changing the partial pressure of carbon dioxide. Here, again, we shall follow Van Slyke.[88] In figure 20, A approximately represents the composition of oxygenated human blood when the value of pH_s is 7.8. The conditions are those defined by equations 3, 4, 5, and 6. In other words, ionic neutrality exists in cells and in serum, osmotic equilibrium exists between cells and serum, and the values of r for chloride and bicarbonate ions are equal. B represents the changes in the cells following increase of the pressure of carbon dioxide to the value where pH_c becomes 6.6—the isoelectric point of oxyhemoglobin. Now all the base which was bound by hemoglobin has become bicarbonate,

[87] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 765 (1923).

[88] D. D. Van Slyke, *Factors Affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body,* pp. 18-20, Philadelphia and London, 1926.

and HCO_3^- has completely replaced Hb^-. Thus both the osmotic equilibrium and the Gibbs-Donnan equilibrium must be disturbed. C represents the effect of the exchange of chloride ion for bicarbonate ion between the two phases to restore to equality the values of r for chloride and bicarbonate ions. But osmotic equilibrium is still unattained. Finally D represents the condition of equilibrium, after the movement of water has accomplished the osmotic readjustment. Needless to say, these processes are simultaneous, not successive, but such graphical analysis, though for this and other reasons not quite accurate, greatly facilitates the understanding of the complex reality.

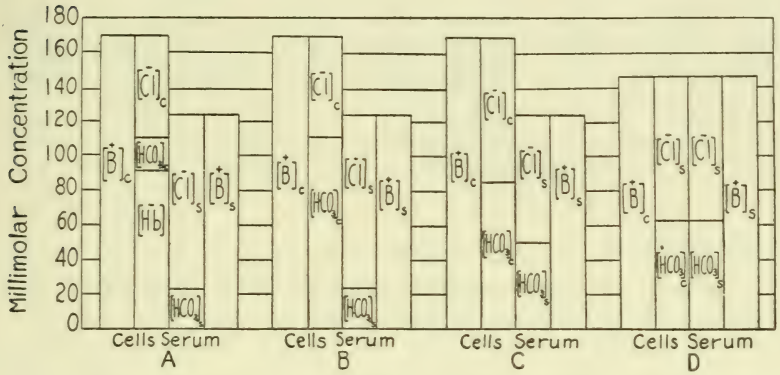


Fig. 20

Readjustment of the Heterogeneous Equilibrium

When the small quantities of base bound by serum protein and the small osmotic effect of hemoglobin are taken into account, the conditions are as represented in figure 21, which is based upon experimental observations of Van Slyke, Wu, and McLean. Such considerations may be extended to take account of still wider variations of condi-

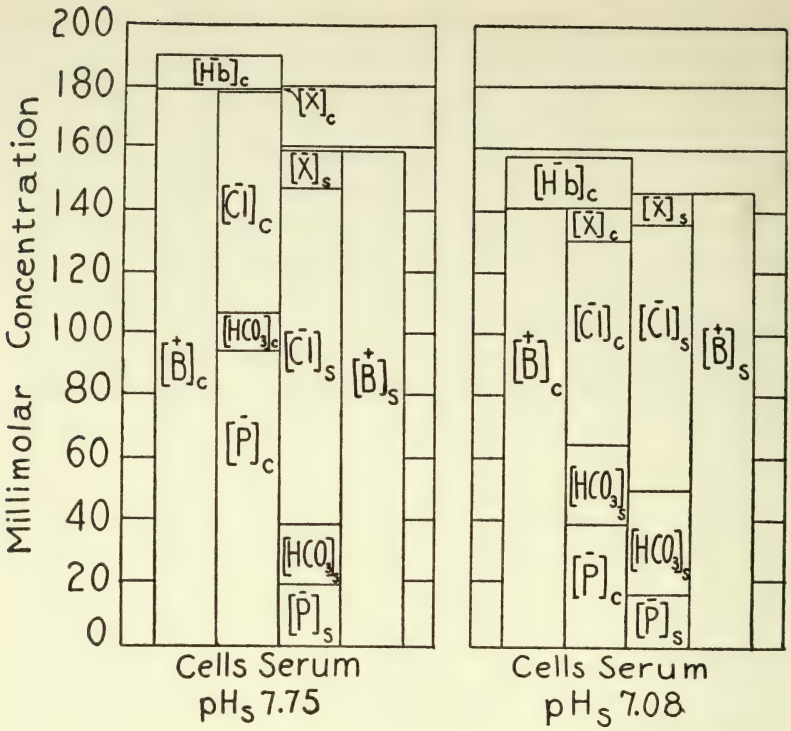


Fig. 21

Blood: The Heterogeneous Equilibrium. Exact Description

tions. Proceeding in this manner, Van Slyke, Wu, and McLean have obtained an equation,[89]

$$r = 1 - \frac{(BP)_c + (Hb)_c}{2(B)_c - (BP)_c + (Hb)_c} + \frac{(BP)_s}{2(B)_s - 2(BP)_s}, \qquad (11)$$

which approximately defines the values of r for all conditions in normal mammalian blood. In this equation it should be noted that concentrations are expressed in units per kilogram of whole blood. This equation yields figure

[89] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 776, 785 (1923).

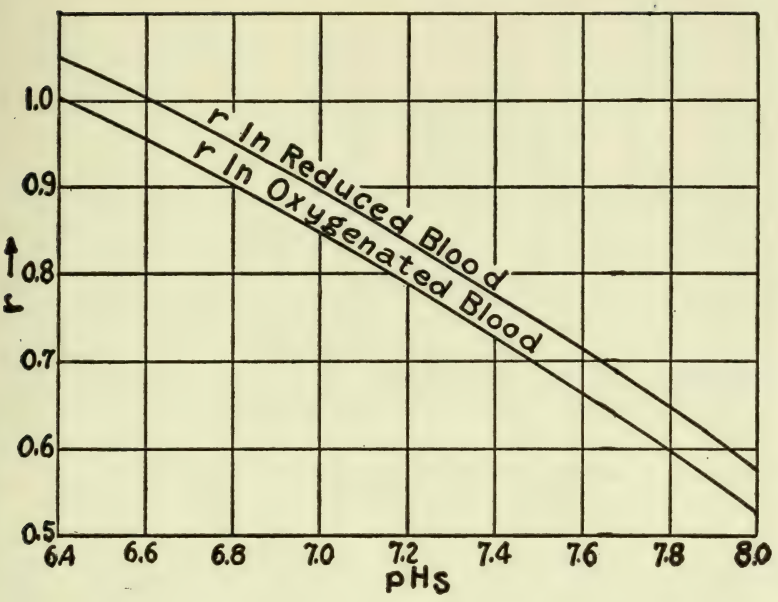


Fig. 22

Values of r and of pH_s: *Calculated*

22, on which values of r for oxygenated and reduced blood are represented as functions of pH_s.

These results of an approximate theoretical treatment of the heterogeneous ionic equilibrium have been experimentally tested by Van Slyke and his collaborators,[90] and by my collaborators at the Massachusetts General Hospital.[91] In view of the complexity of the phenomena and the large number of simplifying assumptions, the agreement between fact and theory is satisfactory. A few data, for comparison with the theory, are given in figure 23. The most striking discrepancy between fact and theory is to be seen in the difference between the values of r for chlo-

[90] Van Slyke, Hastings, Murray, and Sendroy, *Journal of Biological Chemistry,* LXV, 701 (1925).

[91] Bock, Dill, Hurxthal, Lawrence, Coolidge, Dailey, and Henderson, *Journal of Biological Chemistry,* LXXIII, 749 (1927).

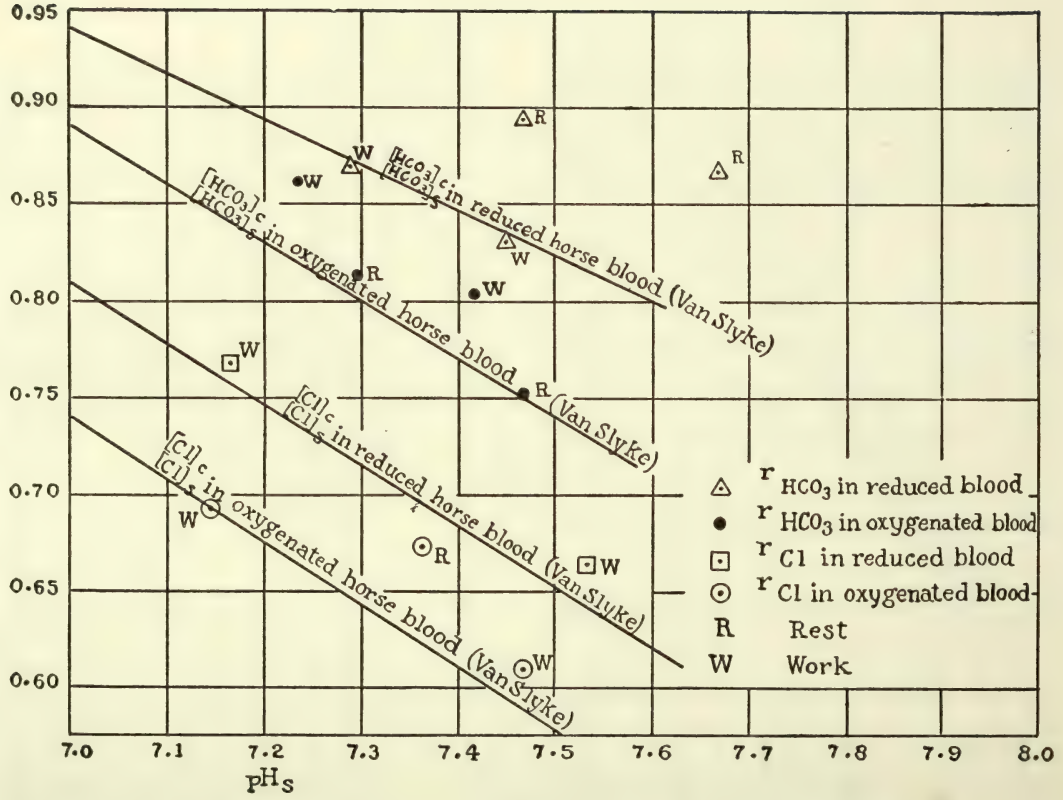


Fig. 23

Values of r and of pH_s: *Observed*

ride and bicarbonate ions. Taking all observations into account, we have, roughly,

$$\frac{[Cl^-]_c}{[Cl^-]_s} = 0.8\,\frac{[HCO_3^-]_c}{[HCO_3^-]_s}, \qquad (12)$$

a result which suggests that the most important unknown factor in this equilibrium may be the activity coefficients of the two ions in the two phases.

A similar theoretical analysis based upon the same assumptions leads Van Slyke, Wu, and McLean to an expression for the osmotic equilibrium between cells and plasma in terms of water distribution and the variables above considered:[92]

$$(H_2O)_c = (H_2O)_B\,\frac{2(B)_c - (BP)_c + (Hb)}{2(B)_B - (BP)_s - (BP)_c + (Hb)}, \qquad (13)$$

and this may also be represented graphically in convenient form as in figure 24. Here cell volume is given as a function of pH_s and of concentration of hemoglobin. In this case, also, the facts experimentally determined agree approximately with the theory.

It can hardly have escaped the reader that such theoretical considerations must also find application to the exchanges between blood plasma and the cells of the body and to exchanges between blood plasma and other body fluids, such as lymph, cerebro-spinal fluid, edema fluid, and urine. While exchanges between blood or lymph and protoplasm have not yet been brought within the scope of our investigations, the relations between plasma and other fluids are in some respects well defined. Thus it has been shown by Mestrezat[93] that cerebro-spinal fluid when dialyzed against serum suffers no measurable change in composition, and the same observation has been made by

[92] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry*, LVI, 781 (1923).

[93] W. Mestrezat, *Le liquide céphalo-rachidien normal et pathologique*, Paris, 1912.

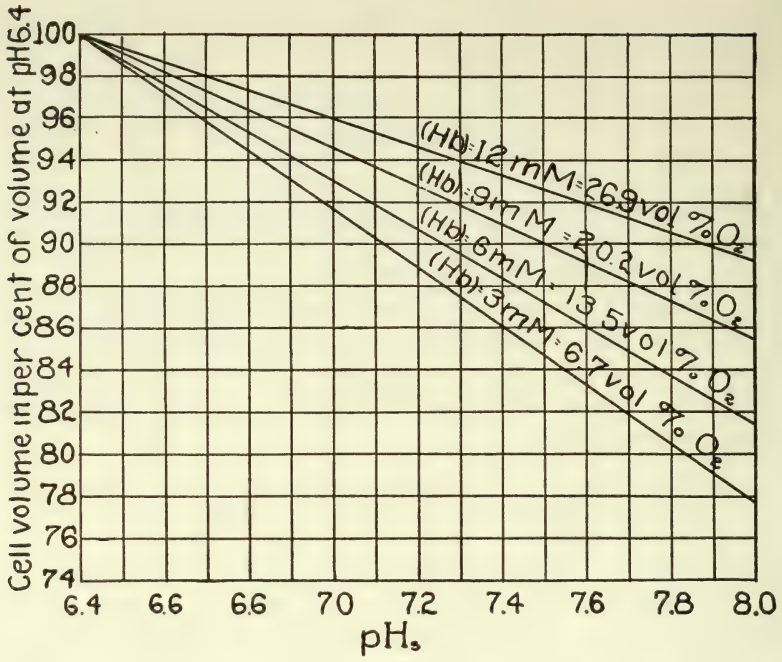


Fig. 24

Values of Cell Volume and of pH_s

Loeb, Atchley, and Palmer[94] for the two-phase system comprising edema fluid and serum. There is no evidence to show that any of the ions of serum are unable to pass over into the other fluids, and it is certain that sodium, as well as the anions can do so. Hence, it is apparent that a condition nearly or quite indistinguishable from equilibrium exists between blood and some of the other fluids of the body, and that this equilibrium is not subject to the condition restricting the movement of cations between the two phases which exists in blood. Nevertheless, there are considerable differences in composition between blood plasma, on the one hand, and cerebro-spinal fluid or edema fluid, on the other hand. Proteins make up about seven

[94] Loeb, Atchley, and Palmer, *Journal of General Physiology,* IV, 591 (1921-1922).

per cent of the whole mass of plasma, but ordinarily less than four per cent of the mass of edema fluid, where they are often present only in traces. The composition of cerebro-spinal fluid is similar.

Here we may consider the case where proteins are absent from the second phase and, as a rough approximation, treat electrolytes as if they consisted exclusively of the univalent salt BA. In serum, the concentration of BP is about 12 milliequivalents per liter. Hence, by an analysis similar to that which leads to equations 8 and 9, the concentration of B^+ in plasma must be greater than that in edema fluid and the concentration of A^- in serum must be less than that in edema fluid by one-half the concentration of BP, or six millimoles per liter. Also, in accordance with the Gibbs-Donnan law we have

$$\frac{(A^-)_s}{(A^-)_E} = \frac{(B^+)_E}{(B^+)_s} = r.$$

For blood serum, (A^-) is approximately 150 millimoles per liter, and (B^+) approximately 162 millimoles per liter. Therefore,

$$\frac{150}{156} = \frac{156}{162} = r,$$

an expression in which the approximate character of the discussion is obvious. From these equations we have,

$$r = 0.96.$$

The observations of Salvesen and Hastings[95] on a case in which edema fluid was nearly free from protein are given in the following table:

TABLE 6.

	Serum	Fluid	r
(HCO_3^-)	29.3	30.4	0.964
(Cl^-)	116.8	120.0	0.973
(Na^+)	166.8	156.2	0.937
Mean			0.96

[95] *Cf.* D. D. Van Slyke, *Factors Affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body,* Philadelphia and London, 1926.

Other observations yield similar values of r, and the agreement between facts and theory is satisfactory. In the case of cerebro-spinal fluid, there seems to be a somewhat less close agreement between theory and observation, which may be due to a less close approach to equilibrium, or perhaps to unsuitable methods of experimentation. But there can be little doubt that the Gibbs-Donnan theory explains all the important features of the case.

Under these circumstances, however, the distribution of water presents a more complex problem, for here there arises, at least in simple cases, a tendency to a great increase in the volume of the phase containing the non-diffusible substance. In this manner remarkable osmotic phenomena, which have been studied by Proctor and Wilson[96] and by Loeb,[97] may occur. Within the blood this is not the case, since the restriction of movement of base between cells and plasma permits equilibrium, without the intervention of mechanical pressure or surface tension to check the movement of water. But possibly in the exchanges between plasma and other fluids, and certainly in simple artificial systems, osmotic phenomena which can only be balanced by significantly large mechanical pressures play a part. It was this fact which interfered with my early experiments on equilibrium in heterogeneous protein systems.

The explanation of the fact is as follows: Let there be a two-phase system containing in phase I the diffusible ions Na^+ and Cl^- and the non-diffusible ion P^-, in phase II only Na^+ and Cl^-. Then we have

$$r = \frac{(Cl^-)_I}{(Cl^-)_{II}} = \frac{(Na^+)_{II}}{(Na^+)_I},$$

therefore $\quad (Na^+)_I \times (Cl^-)_I = (Na^+)_{II} \times (Cl^-)_{II}.$

[96] Proctor and Wilson, *Journal of the Chemical Society,* CIX, 307 (1916).

[97] J. Loeb, *Proteins and the Theory of Colloidal Behavior,* New York and London, 1922.

Also $(Na^+)_I = (Cl^-)_I + (P^-)_I,$

therefore $(Na^+)_I > (Cl^-)_I.$

Moreover $(Na^+)_{II} = (Cl^-)_{II}.$

But since the perimeter of a square is less than that of any other rectangle of equal area, we have

$$(Na^+)_I + (Cl^-)_I > (Na^+)_2 + (Cl^-)_2,$$

and, *a fortiori,*

$$(Na^+)_I + (Cl^-)_I + (P^-)_I > (Na^+)_{II} + (Cl^-)_{II}.$$

Or, in words, the concentration of phase I is greater than that of phase II. Therefore there is a tendency for water to pass from phase II to phase I.

In accordance with this consideration, Van Slyke[98] estimates the mechanical pressure necessary to establish equilibrium between blood plasma and protein-free lymph as 25 mm., of which one-fifth is due to the unequal distribution of diffusible ions in question, and four-fifths to the osmotic effect of the plasma proteins. This is of the same order as measurements by Schade and Claussen,[99] and by Hastings.[100] Thus a study of heterogeneous equilibrium by means of the Gibbs-Donnan theory explains in a more ample manner the process of lymph formation as described by Starling. The problems of edema have also been clarified by the applications of the laws of heterogeneous equilibrium and through studies of the composition of blood plasma in pathological conditions. But at this point much remains to be done before the subject can be expected to yield to systematic rational analysis.

[98] D. D. Van Slyke, *Factors Affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body,* chap. II, Philadelphia and London, 1926.

[99] Schade and Claussen, *Zeitschrift für Klinische Medizin,* C, 363 (1924).

[100] D. D. Van Slyke, *Factors Affecting the Distribution of Electrolytes, Water, and Gases in the Animal Body,* p. 37, Philadelphia and London, 1926.

Progress is also being made in the application of the principles with which we have dealt in this chapter to the study of the formation of cerebro-spinal fluid and of urine. These questions we can but note in passing, since our problem is a restricted one.

Up to this point we have been concerned with a mere analytical description of the physico-chemical changes which occur in blood during the respiratory cycle. This description is now completed, or at least concluded. Presented even in this summary and simplified form the facts must seem bewildering in their complexity; yet the interrelations between them are even more difficult to grasp. Each of the processes which we have studied involves familiar problems of physical chemistry, but their interaction, though no less physico-chemical, leads to a physiological problem, that of their harmonious integration.

CHAPTER VI

THE PHYSICO-CHEMICAL SYSTEM

THE parts of which the respiratory activity of blood is composed are now before us. They permit, as we have seen, a clear understanding of the conditions and reactions of the components of the physico-chemical system. But with the increase of our knowledge it has become more and more difficult to conceive the harmonious interaction of these parts in the complex physiological process. Or, to speak precisely, it has become increasingly difficult to return from the study of our manifold physico-chemical abstractions to the more concrete physiological reality. This task, which we must now undertake, is a difficult one, as is demonstrated by the frequent failure of those who have established the facts to understand their wider significance. Without the aid of a mathematical method it is indeed an impossible task.

We commenced the present study with a postulate that the components of blood of which the treatment is necessary and sufficient for the description of the respiratory function are eight:—H_2O, CO_2, O_2, HCl (including HX), BOH_s, BOH_c, P_s, P_c. The grounds for setting up this postulate have now been fully explained. It was tacitly assumed in my early work[101] and explicitly adopted by Van Slyke, Wu, and McLean.[102] Like all simplifying assumptions it is justified by its success, or by the conven-

[101] L. J. Henderson, *Ergebnisse der Physiologie,* Jahrgang VIII, p. 254 (1909); L. J. Henderson, *Journal of Biological Chemistry,* VII, 29 (1909); Spiro and Henderson, *Biochemische Zeitschrift,* XV, 114 (1909).

[102] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 765 (1923).

ience that it affords, and will remain in use until convenience prescribes some change.

In accordance with this postulate and with Gibbs's theory of heterogeneous equilibrium we have, for any of the physiological variables like total carbonic acid, total oxygen, pH_s, pH_c, r, or cell volume, an equation of the type:

$$\text{Total } CO_2 = \Phi \{ p, T, (H_2O), pCO_2, pO_2, (HCl), (BOH)_s, (BOH)_c, (P)_s, (P)_c \}. \quad (1)$$

Here p, or total pressure, may be regarded as constant and, if we restrict our studies to normal mammals, T is also constant. Thus, the number of independent variables is reduced by two, with the result that we obtain the relation:

$$\text{Total } CO_2 = F \{ (H_2O), pCO_2, pO_2, (HCl), (BOH)_s, (BOH)_c, (P)_s, (P)_c \}, \quad (2)$$

and similarly for the other physiological variables.

In the case of total carbonic acid we have already reviewed evidence which shows that this quantity varies not only with carbon dioxide and oxygen partial pressures, *i.e.*, with pCO_2 and pO_2, but also with the concentrations of cell and serum proteins, *i.e.*, with $(P)_c$ and $(P)_s$, with the amount of available base, *i.e.*, with $(BOH)_s$, $(BOH)_c$, and (HCl), and finally with the state of the heterogeneous equilibrium between cells and plasma, *i.e.*, with (H_2O) and (HCl). Also the absorption curve of carbonic acid depends upon the activity coefficients of H_2O, of CO_2, of H_2CO_3, and of the ion HCO_3^-, all of which vary in case (H_2O) and (HCl) vary sensibly. Thus it may be seen that a complete description of the carbon dioxide absorption of mammalian blood must, in fact, involve at least the eight independent variables above specified. Indeed, if it should later appear that the acid groups of the proteins differ in any important degree from species to species, this number might even be

increased.[103] But no evidence exists to show that more than these eight variables need now be considered.

We have also seen that total oxygen, hydrogen ion concentration, volume of cells, the Donnan r, and the other variables which have thus far engaged our attention are all functions of total carbonic acid. Therefore they too are functions of the eight independent variables. In all these cases, also, there is no evidence that other independent variables need to be considered.

The comparative study of blood has not yet made great progress, and the explicit forms of all of these equations in eight independent variables remain unknown. In general, existing knowledge of the facts has been acquired by the study of blood which has been removed from the body and in which the masses of the components H_2O, HCl, BOH_s, and BOH_c, P_s and P_c are all constant, though varying from specimen to specimen, while only pCO_2 and pO_2 remain as independent variables. If temperature also is held constant, we have, in contrast with the case of mammalian blood in general, for each specimen of blood relatively simple equations of the type

$$\text{Total } CO_2 = f_1(pCO_2, pO_2), \tag{3}$$

$$\text{Total } O_2 = f_2(pCO_2, pO_2). \tag{4}$$

And these two equations are, in fact, the analytical expression of the discoveries of Bohr, Hasselbalch, and Krogh[104] and of Christiansen, Douglas, and Haldane.[105]

The case of the acid-base equilibrium is not different from those of the absorption of oxygen and of carbon dioxide. Here we have (equation 6, page 42).

$$[H^+]_s = k' \frac{c \cdot pCO_2}{[BHCO_3]_s}.$$

[103] The experience of Van Slyke's laboratory shows that differences in the hemoglobins of different mammalian species are small but measurable.

[104] Bohr, Hasselbalch, and Krogh, *Skandinavisches Archiv für Physiologie,* XVI, 411 (1904).

[105] Christiansen, Douglas, and Haldane, *Journal of Physiology,* XLVIII, 244 (1914).

But we may deduce from equation 3 that the quantity $[BHCO_3]_s$ is a function of pCO_2 and of pO_2. Therefore, we have also

$$[H^+]_s = f_3(pCO_2, pO_2). \tag{5}$$

Again, according to Van Slyke, Wu, and McLean, the Donnan r, as well as v, the volume of the red cells, is a function of hydrogen ion concentration and of the degree of oxygenation of hemoglobin, therefore, since the two latter variables are both functions of pCO_2 and of pO_2 (equations 4 and 5), we have

$$r = f_4(pCO_2, pO_2), \tag{6}$$

$$v = f_5(pCO_2, pO_2), \tag{7}$$

and similarly for all the other physiological variables.

The five equations, 3, 4, 5, 6, and 7, state as abstractly as possible the facts discussed in the preceding lectures. They include seven variables—pCO_2, pO_2, total carbonic acid, total oxygen, the hydrogen ion concentration, and the quantities r and v—one less than the number of components of the system, and they suffice for a complete approximate characterization of any specimen of mammalian blood.

If it were not for certain simplifying assumptions such as that of a general law defining the value of r, with or without constant differences for the different anions, and that of osmotic equilibrium, the case would be different. The necessary equations would then be six in number, like the number of components of constant mass, and the total number of variables would be eight. It seems possible that another equation defining independent variations in the values of the several r's may soon be necessary and, in that case, the number of equations will, in fact, be six, the number of variables eight.

It is evident that if we possessed complete information concerning the activities of each of the six components of constant mass in terms of the two variable components

we could deduce from this information all necessary information concerning the physiological activity of blood. But theoretical treatment of the system has not reached the necessary stage of perfection to make this possible. Thus the choice of the five variables, total oxygen, total carbonic acid, hydrogen ion concentration of serum, the Donnan r, and cell volume, is an arbitrary one, or rather a result of the successive steps in the development of the subject. Within limits, some other choice of seven variables would also serve, but the present choice is convenient and has been proved to be sufficient, with the exception of the possible independent variations of the values of r just mentioned.

It is hardly necessary to say that the choice of the concentrations of the components as variables, though theoretically an obvious one, would be very inconvenient in biological studies. But any choice must be such that by elimination among the equations, including the equations defining the chosen variables as functions of the components, it shall be ideally possible to obtain five, or, if the component H_2O be included, six equations containing only the potentials of the seven or eight components as variables.

To sum up, the facts now known may be represented by a series of equations which differ more or less for each specimen of blood. The experimentally determined equations are as follows:

$$\begin{aligned}
\text{Total } CO_2 &= f_1(pCO_2, pO_2),\\
\text{Total } O_2 &= f_2(pCO_2, pO_2),\\
[H^+]_s &= \phi_3(pCO_2, [BHCO_3]_s),\\
r &= \phi_4([H^+], [HbO_2]),\\
v &= \phi_5([H^+], [HbO_2]).
\end{aligned}$$

But the last three equations may all be transformed so as to yield the following set of five equations:

$$\text{Total } CO_2 = f_1(pCO_2, pO_2), \tag{3}$$

$$\text{Total } O_2 = f_2(pCO_2, pO_2), \tag{4}$$

$$[H^+]_s = f_3(pCO_2, pO_2), \quad (5)$$

$$r = f_4(pCO_2, pO_2), \quad (6)$$

$$v = f_5(pCO_2, pO_2). \quad (7)$$

When the facts are presented in this form, which states a mere truism, the problem begins to take shape. Evidently each of these five equations is a partial description of blood, while the five together include, in part explicitly and in part implicitly, a complete description of the processes involved in the respiratory function. Thus a single equation taken by itself can but mislead us concerning the totality of the system. So it was that when years ago Barcroft completed his studies which defined the relation between oxygenation of blood, oxygen pressure, and carbon dioxide pressure,[106] and I explained the condition of the acid-base equilibrium,[107] each of us not unnaturally supposed that the problems we had studied were solved. But this was true only in a very restricted sense, for other variables were involved in the phenomena and these variables were not, as is so often the case in the highly abstract researches of the physicists and of the chemists, theoretically irrelevant. On the contrary, they were no less to the point than the subjects of our investigations. We were studying different aspects of the same thing, but we did not recognize the fact, for the nature of the phenomenon escaped us.

Even the five equations, 3, 4, 5, 6, and 7, state only a small part of what may be deduced from them. There are, in fact, $\frac{7 \times 6 \times 5}{3 \times 2 \times 1} = 35$ combinations among seven objects, taking three at a time. Therefore, there exist 35 analogous expressions describing the respiratory changes in blood and it was by chance that five in particular were first discovered.

[106] J. Barcroft, *The Respiratory Function of the Blood,* 1st ed., Cambridge, England, 1914.

[107] L. J. Henderson, *Ergebnisse der Physiologie,* Jahrgang VIII, p. 254 (1909).

The nature of the case may be illustrated by means of an example taken from elementary mathematics. Let us consider the five equations:

$$z = x + y,$$
$$u = x - y,$$
$$v = x + 2y,$$
$$u = x - 2y,$$
$$r^2 = x^2 + y^2.$$

Each of these equations corresponds to a contour line chart which may be readily constructed. For example, figure 25 is such a geometrical representation of the equation $z = x + y$ and figure 26 of the equation $u = x - y$.

Also it is easy to obtain from these five equations 30 others, each in three variables. For instance, by addition of the first and second, we have

$$z + u = 2x.$$

Next it may be noted that two or more contour line charts, provided they have the same Cartesian coördi-

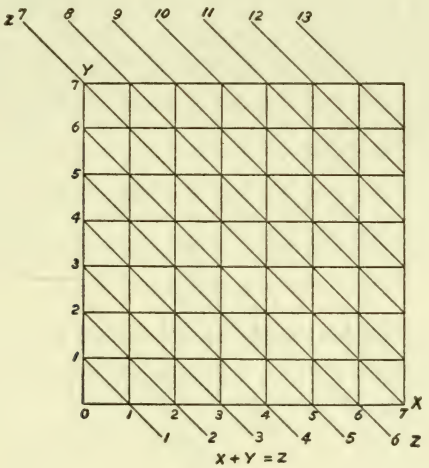


Fig. 25

Contour Line Chart

nates, may be combined, just as a geological map may be superposed on a topographical map, following that if desired with a political map, an ethnographical map, and so on indefinitely.

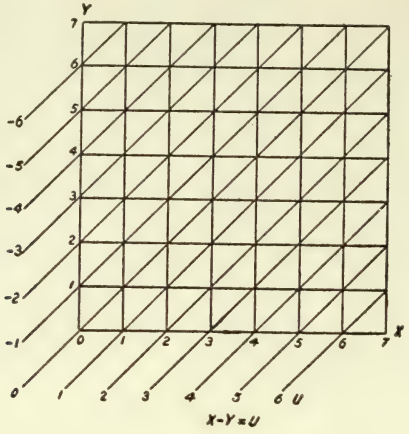


Fig. 26

Contour Line Chart

Figure 27 is the result of such a combination of figures 25 and 26. It expresses all that is expressed by these two figures and, in addition, whatever may be deduced from them when both are known. In other words, it is the expression of four equations:

$$z = x + y,$$
$$u = x - y,$$
$$z + u = 2x,$$
$$z - u = 2y.$$

Nothing is simpler than to continue this process, first constructing contour line charts for the three remaining equations of the original five:

$$v = x + 2y,$$
$$u = x - 2y,$$
$$r^2 = x^2 + y^2,$$

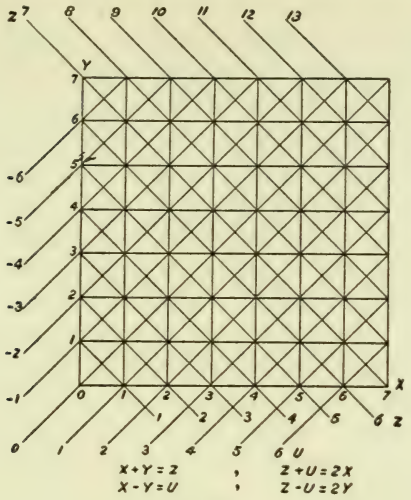


Fig. 27

Cartesian Nomogram

and then superposing these upon figure 27. The resulting Cartesian nomogram, the representation of which presents no particular interest, must evidently define at once all that the 35 contour line charts or the 35 equations in three variables can yield.

On such a figure it is in general possible, when values of two variables are given, to obtain those of all the others. Thus, from figure 27, given $z = 7$ and $u = 1$, we read $x = 4$ and $y = 3$, a result that can be obtained analytically only by solving the two simultaneous equations,

$$x + y = 7,$$
$$x - y = 1,$$

and that can not be obtained directly from figures 25 and 26. Such use of figure 27 depends upon the fact that the position of a point in a plane is determined by two coördinates, while on such a nomogram as figure 27 every point has several coördinates.

We shall now turn from this trivial example to the analogous but more difficult problem of the nomographic representation of the facts considered in the preceding chapter. The construction of a complete nomogram may be conveniently begun with the help of the oxygen and carbon dioxide dissociation curves. For the present purposes, we have at our disposal data concerning these curves and other necessary data obtained from studies of the blood of T.J.F., a patient suffering from pernicious anemia. The measurements were all made on one day, March 23, 1927, at a time when the feeding of liver during a period of two months had restored the blood to a nearly normal condition. The amount of hemoglobin and the number and volume of red cells are, indeed, in the lower ranges of normal variations, but in no sense pathologically low. The red cells, possibly as a result of the presence of nuclear material, are somewhat different in composition from those of a normal man and there are other slight departures from the normal which, however, will cause no inconvenience at this stage of our exposition. This case is chosen, first, because it is desirable to demonstrate the method that may be employed in obtaining a complete description of the blood with but a small number of experimental observations, and, secondly, because many of the data appear to be, by good fortune, somewhat more consistent than is often the case when work has been done hastily and observations are few.

As a beginning of a synthetic treatment of the blood of T.J.F. on March 23, we may take the experimental data which give the total carbonic acid content of whole blood and of true plasma, equilibrated against different pressures of carbon dioxide and oxygen. These data are collected in table 7.

TABLE 7.

Experimental data for carbon dioxide dissociation curves of T.J.F. on March 23, 1927.

Remarks	pCO_2	pO_2	Calculated HbO_2	Total CO_2 Whole blood	Total CO_2 True plasma
	mm. Hg	*mm. Hg*	*per cent saturation*	*vols. per cent*	*vols. per cent*
Arterial blood; first sample ..	38.5	air	100	48.70	
Arterial blood; second sample	38.7	air	100	49.90	
Venous blood ..	20.6	air	100	38.19	45.52
				39.20	
Venous blood ..	100.0	air	100	67.82	74.37
Venous blood ..	15.7	5.2	10	37.95	43.75
Venous blood ..	44.8	9.5	12	54.66	62.35
Venous blood ..	82.0	15.2	13	66.72	74.82

They have been plotted on figure 28, after applying a small linear correction to the observations on partially reduced blood, in order to obtain values corresponding to complete reduction. It will be remembered that at constant hydrogen ion concentration equal changes in oxygenation (per cent saturation of HbO_2) are accompanied by equal changes in base bound by hemoglobin. Also, equal small changes in hydrogen ion concentration are accompanied by equal small changes in base bound by hemoglobin. Finally, changes in base bound by hemoglobin are equal, other things being equal, to changes in bicarbonate.

On figure 28 curves corresponding to completely oxygenated and completely reduced whole blood and true plasma have been drawn. These curves have been chosen in conformity with three conditions: First, they must fit the data as well as possible. Secondly, they must have the form which long experience proves to be that of all carbon dioxide dissociation curves of human blood. Thirdly, all curves must have approximately the same curvature

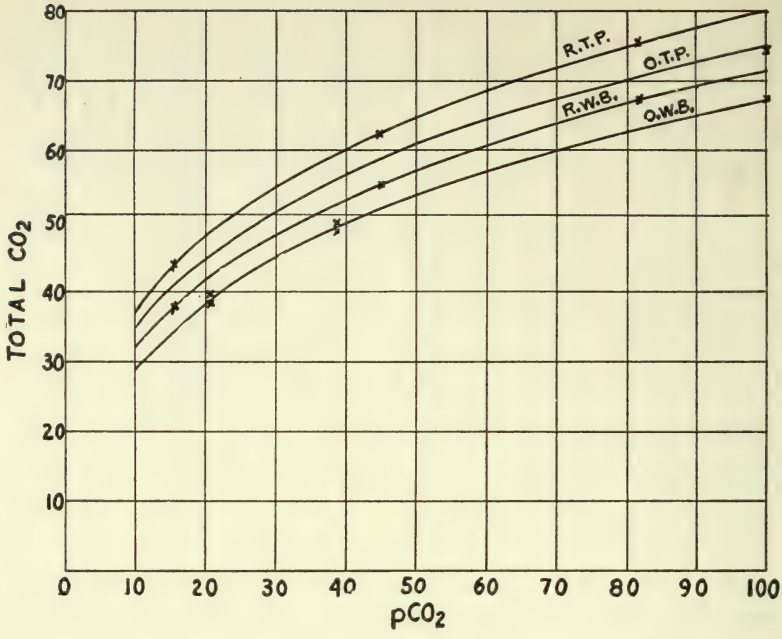


Fig. 28

Carbon Dioxide Dissociation Curves of Whole Blood and True Plasma

at corresponding points throughout a wide range of carbon dioxide pressures, a relation which is known to hold for all carbon dioxide dissociation curves of the same specimen of blood.

In case difficulty is encountered in thus constructing smooth best-fitting curves, recourse may be had to the use of a graph of log pCO_2 against log total CO_2. In this case all curves are known to be approximately straight lines above carbon dioxide pressures of 20 to 30 mm. and for the same specimen of blood these lines are nearly parallel, but ordinarily slightly convergent as carbon dioxide pressures increase. Figure 29 is such a representation of the corrected data of table 7.

TABLE 8.

Smoothed data from the carbon dioxide dissociation curves of T.J.F. on March 23, 1927.

	Plasma	Whole blood	Oxygenated whole blood		Reduced whole blood		Oxygenated true plasma			Reduced true plasma		
pCO_2	H_2CO_3	H_2CO_3	Total CO_2	$BHCO_3$	Total CO_2	$BHCO_3$	Total CO_2	$BHCO_3$	pH_s	Total CO_2	$BHCO_3$	pH_s
	vol.	*vol.*	*vol.*	*vol.*	*vol.*	*vol.*	*vol.*	*vol.*		*vol.*	*vol.*	
mm. Hg	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	
10	0.68	0.63	29.00	28.37	32.10	31.47	34.70	34.02	7.819	37.00	36.32	7.847
20	1.37	1.26	38.35	37.09	41.85	40.59	44.80	43.43	7.621	48.00	46.63	7.652
30	2.05	1.88	44.90	43.02	48.30	46.42	51.40	49.35	7.501	55.05	53.00	7.532
40	2.74	2.51	49.80	47.29	53.41	50.92	56.60	53.86	7.413	60.70	57.96	7.445
50	3.42	3.14	53.70	50.56	57.35	54.21	60.90	57.48	7.345	65.10	61.68	7.376
60	4.10	3.77	57.05	53.28	61.00	57.23	64.40	60.30	7.287	68.80	64.70	7.318
70	4.79	4.40	60.00	55.60	63.95	59.55	67.50	62.71	7.237	72.00	67.21	7.267
80	5.47	5.02	62.90	57.68	66.85	61.70	70.20	64.73	7.192	74.90	69.43	7.224
90	6.16	5.65	65.15	59.50	69.20	63.55	72.90	66.74	7.153	77.70	71.54	7.184
100	6.84	6.28	67.48	61.20	71.60	65.32	75.05	68.21	7.119	80.10	73.26	7.150

From the curves of figure 28 or figure 29, table 8 is constructed. Some of the columns of that table are obtained directly from the curves, the others by calculation, making use of the relations expressed by the following equations:

$$[H_2CO_3] = pCO_2 \times [H_2O] \times 0.0726, \tag{8}$$

where $[H_2O]_B = 865$ and $[H_2O]_s = 943$,

and $$pH_s = 6.12 + \log [BHCO_3]_s - \log [H_2CO_3]_s. \tag{9}$$

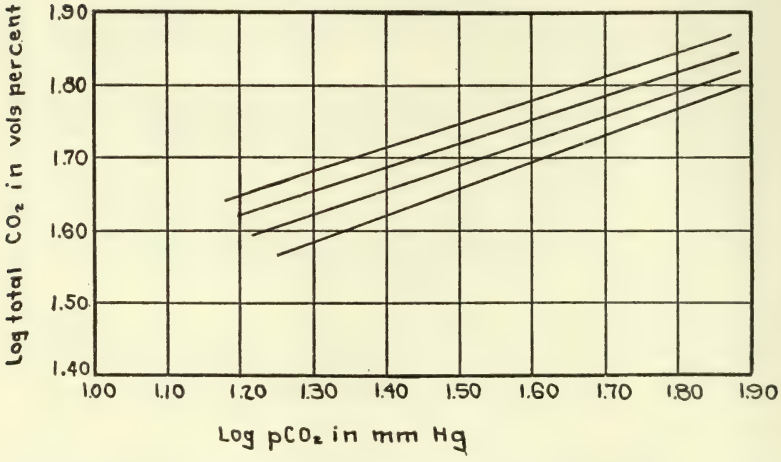


Fig. 29

Logarithmic Carbon Dioxide Dissociation Curves

In equation 8 a small error is introduced if $[H_2O]_s$ be taken as constant. The mean value of this quantity is, as stated, about 943 and, since the variations for the ranges of the table in volume of cells and volume of serum are approximately two per cent, the variation in $[H_2O]_s$ (water per liter of serum) is about ± 0.1 per cent, as the following calculation shows:

Given the weight of one liter serum, 1027 g.; water per liter serum, 943 g.; solids per liter serum, 84 g. Let changes in volume of cells and serum be equivalent to the passage of 10 g. of water per liter blood from cells to plasma or from plasma to cells. Then the extreme values for water per liter serum will be approximately $\frac{953}{1010}$ and $\frac{933}{990}$ or 944 and 942 respectively. Here the variation from the mean is only ± 0.1 per cent, which is negligible in calculating the values of $[BHCO_3]_s$, $[H_2CO_3]_s$, and, therefore, of pH_s.

We turn next to the data concerning equilibrium between blood and oxygen at different pressures of oxygen and of carbon dioxide. These data are given in table 9.

TABLE 9.

Experimental data for oxygen dissociation curves of T.J.F. on March 23, 1927.

pCO_2	pO_2	Total O_2 content	HbO_2 content	Oxygen saturation	Corrected pCO_2	Corrected oxygen saturation	Corrected R
		vol.	*vol.*				
mm. Hg	*mm. Hg*	*per cent*	*per cent*	*per cent*	*mm. Hg*	*per cent*	
19.2	19.9	7.77	7.72	48.3	20.0	48.0	1.084
23.8	34.6	11.73	11.64	72.7	20.0	74.1	0.349
21.2	45.8	14.38	14.27	89.2	20.0	89.8	0.113
23.0	73.5	15.42	15.24	95.2	20.0	95.7	0.045
43.3	25.7	7.58	7.52	47.0	40.0	48.3	1.075
40.6	39.9	12.14	12.04	75.2	40.0	75.4	0.325
38.5	49.5	13.37	13.25	82.8	40.0	82.4	0.214
42.3	65.7	14.59	14.42	90.1	40.0	90.4	0.108
82.8	29.0	5.28	5.21	32.6	80.0	33.4	2.000
84.3	62.9	13.43	13.27	82.9	80.0	83.3	0.200

Here again a small correction must be applied in order to bring the observations to three exact values of pCO_2, *viz.*, 20 mm., 40 mm., and 80 mm.

In this case, also, a linear correction may be made in accordance with the facts as presented in Chapter IV. Practically, however, it is more convenient to apply the small correction obtained from inspection of the oxygen dissociation curves of A.V.B.

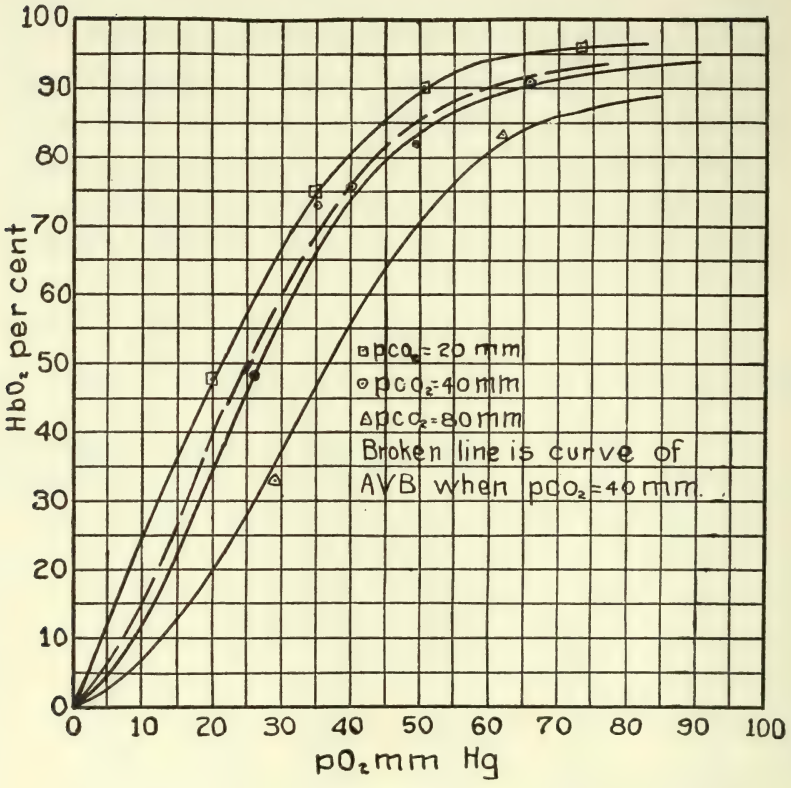


Fig. 30

Oxygen Dissociation Curves

From the data of table 9, figure 30 is constructed. The values of the two last columns are shown as points and curves are drawn to fit these points with the restrictions (1) that they must conform to the known shape of oxygen dissociation curves and (2) that their spacing must correspond to the values represented by all the points. In this case also the use of logarithms simplifies the curves and facilitates the construction. Figure 31 presents this logarithmic transformation. From figure 30 or figure 31 table 10 is constructed.

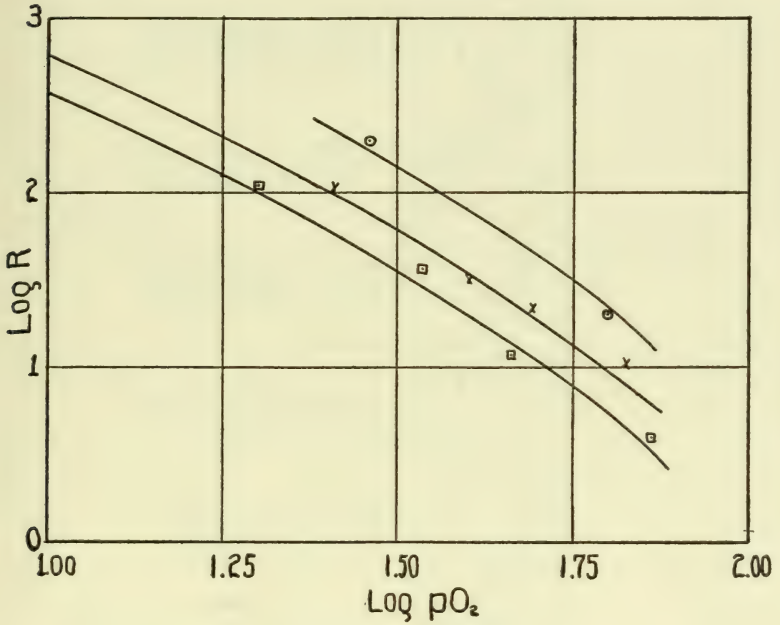


Fig. 31

Logarithmic Oxygen Dissociation Curves

TABLE 10.

Smoothed data from the oxygen dissociation curves of T.J.F. on March 23, 1927.

		TOTAL HbO_2						TOTAL OXYGEN		
Free O_2	pO_2	pCO_2 20 mm.		pCO_2 40 mm.		pCO_2 80 mm.		pCO_2 20 mm.	pCO_2 40 mm.	pCO_2 80 mm.
vol. per cent	*mm. Hg*	*per cent saturation*	*vol. per cent*	*per cent saturation*	*vol. per cent*	*per cent saturation*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
0.01	5	9.0	1.44	5.0	0.80	3.0	0.48	1.45	0.81	0.49
0.03	10	21.6	3.46	13.1	2.10	7.3	1.17	3.49	2.13	1.20
0.05	20	48.0	7.68	35.6	5.70	19.8	3.17	7.73	5.75	3.22
0.08	30	68.5	10.96	57.5	9.20	37.5	6.00	11.04	9.28	6.08
0.11	40	81.4	13.03	73.2	11.72	55.8	8.93	13.14	11.83	9.04
0.14	50	89.0	14.25	84.0	13.45	70.4	11.27	14.39	13.59	11.41
0.16	60	93.3	14.93	89.9	14.39	80.3	12.85	15.09	14.55	13.01
0.19	70	96.0	15.36	93.5	14.96	87.0	13.92	15.55	15.15	14.11
0.22	80	97.8	15.65	95.8	15.33	91.5	14.65	15.87	15.55	14.87
0.27	100	99.0		98.5	15.75	96.0	15.36		16.02	15.63

Next we may undertake a synthetic treatment of the carbonic acid and oxygen dissociation curves of whole blood. From table 8 figure 32 is constructed. Here values

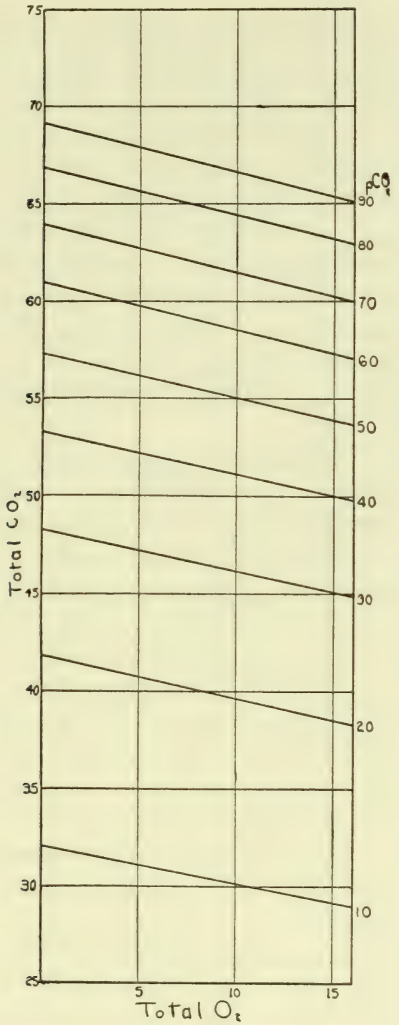


Fig. 32

Transformation of Carbon Dioxide Dissociation Curves

of combined oxygen (volumes per cent of HbO_2) are plotted as abscissas, values of total carbonic acid as ordinates, while values of carbon dioxide pressure appear as contour lines. These lines are straight for reasons above stated.

The next step consists in applying the data of table 10 to figure 32 as follows: Beginning with $pO_2 = 5$ mm. (the first row of table 10) we select the pair of values $pCO_2 = 20$ mm., $HbO_2 = 1.44$ volumes per cent, find the point on figure 32 where the abscissa corresponding to $HbO_2 = 1.44$ volumes per cent cuts the contour line corresponding to $pCO_2 = 20$ mm. and mark it. This process is then repeated for the other pairs of values of HbO_2 and pCO_2 of the first row of the table and the three points thus obtained are joined by a smooth curve. This curve is the contour line for $pO_2 = 5$ mm. The process is now repeated for $pO_2 = 10, 20, \ldots 100$ mm. with the result represented by figure 33.

This is a Cartesian nomogram which completely illustrates the conditions of equilibrium between free and combined, or approximately total, oxygen and free and total carbonic acid, relations incompletely expressed by the original dissociation curves. Because the Cartesian coördinates stand for values of total carbonic acid and approximately total oxygen, while the contour lines represent the two independent variables pCO_2 and pO_2, which are also the physiological variables, the figure is in some respects the most useful of all representations of the blood equilibrium. Accordingly, we shall later return to the consideration of the relations thus expressed.

We may next take account of the values of pH_s. These are given in table 8, having been calculated in the usual manner (equation 9) from the carbon dioxide dissociation curve of true plasma on the above justified assumption of constant water content. The values may be applied to figure 32 without difficulty. The result is figure 34.

Next in order is the question of variation in volume of cells and plasma. Here direct measurements, unless car-

ried out with extreme care, have been thus far liable to large relative errors. It is, therefore, expedient to be guided by theoretical considerations in estimating these

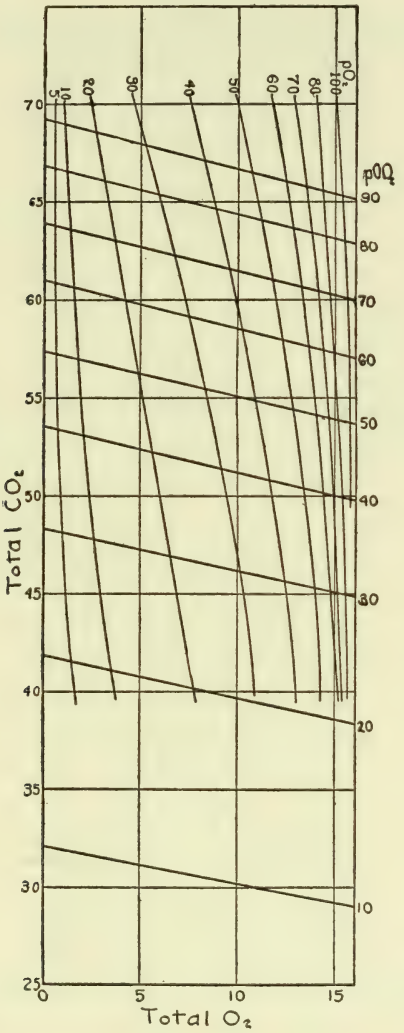


Fig. 33

Synthesis of Carbon Dioxide and Oxygen Dissociation Curves

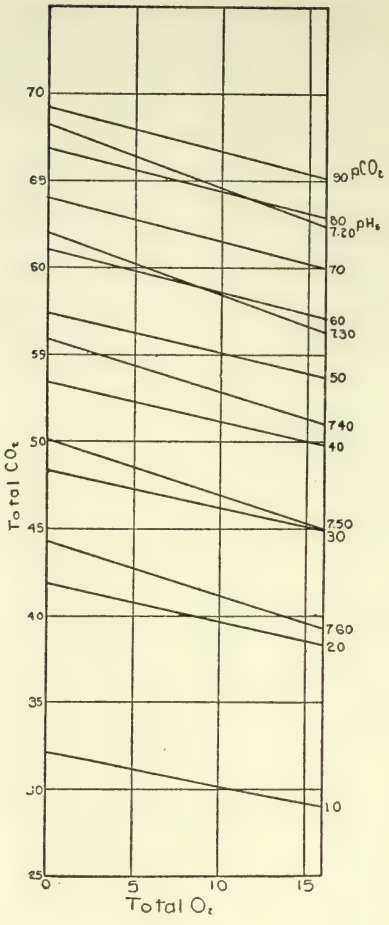


Fig. 34

The Inclusion of Values of pH_s

changes. However, considerable errors in estimation of the relative magnitudes of volume changes produce but slight modifications in the calculated values of other variables and are, therefore, of secondary importance.

For the present case, we have four measurements of cell volume which are collected in table 11 and supple-

mented by the corrected values for completely reduced blood.

TABLE 11.

Cell volumes of the equilibrated blood of T.J.F. on March 23, 1927.

pCO_2	pO_2	HbO_2	pH_s	Cell volume
		per cent		
mm. Hg	*mm. Hg*	*saturation*		*per cent*
20.6	air	100	7.61	36.7
100.0	air	100	7.12	38.1
15.7	5.2	10	7.72	37.1
82.0	15.2	13	7.22	38.3
15.7	0.0	0	7.73	37.2
82.0	0.0	0	7.23	38.3

The values of table 11 for oxygenated and completely reduced bloods are represented as points on figure 35. On this figure theoretical values of volume as a function of

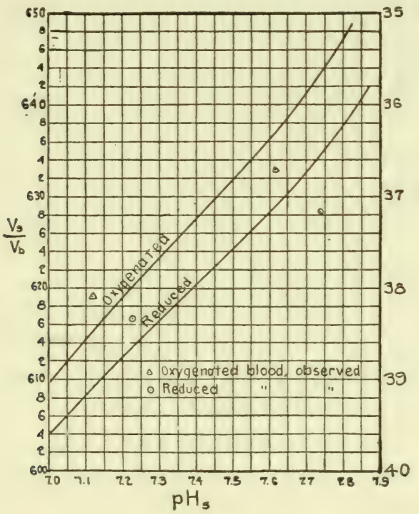


Fig. 35

Values of Cell Volume and pH_s

pH_s obtained from Van Slyke, Wu, and McLean's approximation,

$$\frac{(H_2O)_c}{(H_2O)_B} = \frac{2(B)_c - (BP)_c + (Hb)}{2(B)_B - (BP)_s - (BP)_c + (Hb)},$$

with the use of our observations of the composition of the present specimen of blood, are represented as curves.

We have for this specimen of blood the following measurements:

$(Hb) = 6.8$ millimoles per kilogram,
$(B)_c = 40.8$ millimoles per kilogram,
$(B)_s = 91.6$ millimoles per kilogram,
$(B)_B = 132.4$ millimoles per kilogram,
$(H_2O)_B = 822$ g. per kilogram,
$(P)_s = 40$ g. per kilogram,
$G_B = 1.052$.

Substitution of these values in the above equation yields the relation,

$$(H_2O)_c = 822\,\frac{88.4 - (BP)_c}{271.6 - (BP)_s - (BP)_c},$$

and from this equation, taking account of the known rate of change of the quantity pH_c with change of pH_s, it is possible to calculate values of $(H_2O)_c$ and therefore of $\frac{V_s}{V_B}$ for the present specimen of blood. The values of $\frac{V_s}{V_B}$ differ from those observed with the hematocrit. We shall employ the mean hematocrit reading as a measure of mean cell and plasma volumes, but, in view of the uncertainty concerning the magnitudes of small differences between small numbers of hematocrit readings, the theoretical values for estimating changes in V_c and V_s. The lines on figure 36 are thus drawn. In case errors are thereby introduced through incorrect estimates of BP_c they can be corrected later by a method of successive approximations when, on the basis of the values of B_s and B_c, esti-

mates are made of $(BHCO_3)_c$. On figure 36 the values of figure 35 are applied to the Cartesian background of figure 32 after taking account of the pH_s values of figure 34.

The estimation of $(BHCO_3)_c$ is a simple one. We have already values of total carbonic acid for whole blood and true plasma. From these, concentrations of blood bicarbonate and of serum bicarbonate may be deduced by sub-

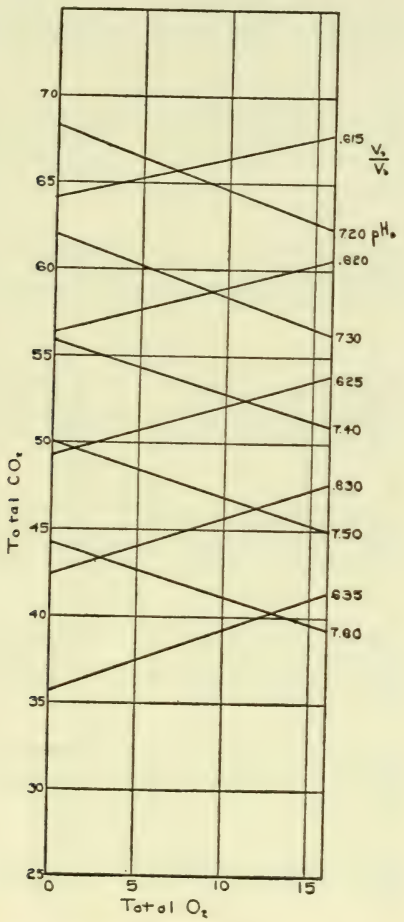


Fig. 36

The Inclusion of Values of Cell Volume

TABLE 12.

r_{HCO_3} for the blood of T.J.F. on March 23, 1927.

A	B	C	D	E	F	G	H	I	J
pCO_2	V_s/V_b	V_c/V_b	$(H_2O)_s$	$(H_2O)_c$	$(BHCO_3)_s$	$(BHCO_3)_c$	F/D	G/E	I/H = *r*
mm. Hg	*per cent*	*per cent*	*cc. per cc. blood*	*cc. per cc. blood*	*vol. per 100 cc. blood*	*vol. per 100 cc. blood*			
				Oxygenated blood.					
10	64.88	35.12	0.6132	0.2518	22.08	6.29	36.00	24.98	0.694
20	63.79	36.21	0.6023	0.2627	27.70	9.39	45.99	35.74	0.777
30	63.20	36.80	0.5964	0.2686	31.18	11.84	52.29	44.09	0.843
40	62.80	37.20	0.5924	0.2726	33.84	13.45	57.11	49.35	0.864
50	62.50	37.50	0.5894	0.2756	35.93	14.63	60.96	53.10	0.871
60	62.25	37.75	0.5869	0.2781	37.54	15.74	63.96	56.60	0.885
70	62.03	37.97	0.5847	0.2803	38.84	16.76	66.40	59.78	0.900
80	61.85	38.15	0.5829	0.2821	39.97	17.71	68.58	62.77	0.915
90	61.69	38.31	0.5813	0.2837	41.01	18.49	70.55	65.20	0.924
100	61.54	38.46	0.5798	0.2852	41.99	19.21	72.41	67.36	0.930
				Reduced blood.					
10	64.01	35.99	0.6045	0.2605	23.25	8.22	38.46	31.56	0.821
20	63.09	36.91	0.5943	0.2697	29.42	11.17	49.50	41.40	0.836
30	62.58	37.42	0.5902	0.2748	33.18	13.24	56.21	48.18	0.857
40	62.22	37.78	0.5866	0.2784	36.07	14.85	61.49	53.36	0.868
50	61.94	38.06	0.5838	0.2812	38.21	16.00	65.45	56.90	0.869
60	61.70	38.30	0.5814	0.2836	39.92	17.31	68.66	61.03	0.889
70	61.50	38.50	0.5794	0.2856	41.34	18.21	71.35	63.77	0.894
80	61.33	38.67	0.5777	0.2873	42.59	19.11	73.71	66.50	0.902
90	61.18	38.82	0.5762	0.2888	43.71	19.84	75.86	68.70	0.907
100	61.04	38.96	0.5748	0.2902	44.72	20.60	77.80	70.98	0.912

tracting the concentrations of free carbonic acid calculated from the values of pCO_2. Concentration of serum bicarbonate multiplied by serum volume then gives serum bicarbonate per liter blood and this quantity subtracted from the concentration of blood bicarbonate gives cell bicarbonate per liter blood. Finally, this quantity divided by cell volume gives the concentration of bicarbonate per liter of cells. When we are at length in possession of the necessary information concerning concentration of bicarbonate per liter of serum, $(BHCO_3)_s$, and per liter of cells, $(BHCO_3)_c$, we may calculate the value of r for the bicarbonate ion, since serum water, $(H_2O)_s$, and cell water, $(H_2O)_c$, are also known from the values of V_s and V_c. The value of this quantity is, in fact, given by the equation,

$$r_{HCO_3^-} = \frac{(BHCO_3)_c}{(H_2O)_c} \times \frac{(H_2O)_s}{(BHCO_3)_s}.$$

The result of these calculations is presented on figure 37,

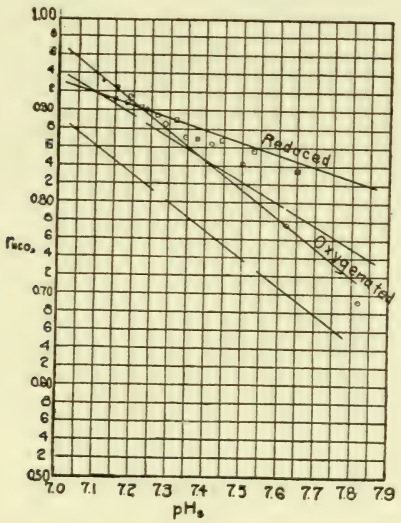


Fig. 37

Values of r and of pH_s

while figure 38 shows the application of the same result to the Cartesian background of figure 32 with the help of the contour lines of pH_s.

The variation in the apparent values of the Donnan *r* for the ions Cl^-, HCO_3^-, and H^+ have been referred to. Such variations may be provisionally ascribed to differences in the activity coefficients of the different substances

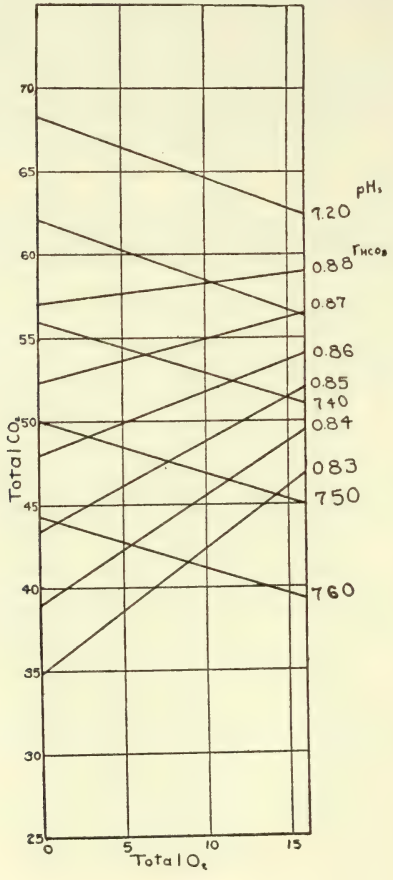


Fig. 38

The Inclusion of Values of r

under different conditions. Experiment gives for these values approximately the following relations:

$$r_{Cl^-} = \frac{5}{4} \cdot r_{HCO_3^-},$$

$$r_{H^+} = \frac{8}{5} \cdot r_{HCO_3^-}.$$

In the present case we have no measurement of r_{H^+} and must, accordingly, adopt the above relation in our further calculations. The values of $r_{HCO_3^-}$ are themselves slightly different from those observed in other cases and in this respect the present data appear to be somewhat less consistent with Van Slyke's approximation from theory than is often the case. This discrepancy is possibly due to the presence of nuclear material in the cells. It produces, however, no significant inconsistency in estimations of other concentrations and our data seem to agree approximately as well with the data of Van Slyke as the latter data do with the theoretical approximations.

The geometrical transformations just described have put us in possession of figures (32, 33, 34, 36, and 38) giving all the other variables (pCO_2, pO_2, pH_s, V and r) as contour lines on a Cartesian background of total carbonic acid and HbO_2. The superposition of these figures will, therefore, yield a Cartesian nomogram similar to that of figure 18, Chapter V. This nomogram presents all the information included in the several contour line charts and in the original data. Such a figure is, however, difficult to read because of the large number of lines. It will, therefore, be convenient at this stage to undertake a transformation to an alignment chart, or nomogram, of the type invented by d'Ocagne. The necessary construction for such a transformation (figure 39) is as follows:[108]

Let Ox and Oy be the axes of a Cartesian nomogram and KL any straight line. Draw two parallel axes Au and

[108] Henderson, Bock, Field, and Stoddard, *Journal of Biological Chemistry,* LIX, 386 (1924).

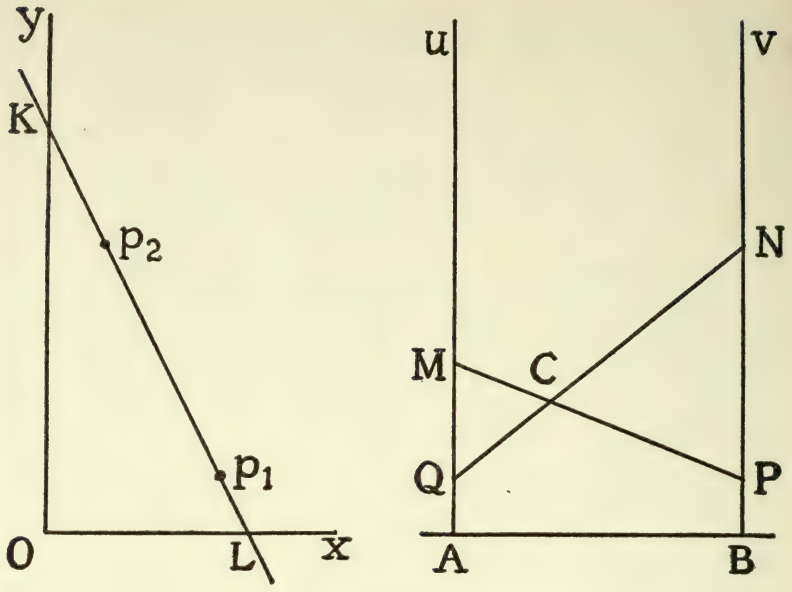


Fig. 39

D'Ocagne's Transformation

Bv. Now read the Cartesian coördinates, x_1, y_1, and x_2, y_2, of any two points, say p_1 and p_2, of the line KL. On Au lay off (taking account of sign) the distance AM $= x_1$ and on Bv the distance BP $= y_1$. Join MP. On Au lay off the distance AQ $= x_2$ and on Bv the distance BN $= y_2$. Join NQ. Then C, the point of intersection of MP and NQ, is the correlative of the line KL. If KL is one of several contour lines, points corresponding to the others may be found by the same method, and, upon joining all these points, a scale of values of the variable, z, corresponding to the contour lines, is obtained.

This process may be repeated for any other family of contour lines, corresponding to values of any other variable, w, on the same Cartesian background. Graduation of Au and Bv to represent values of the variables x and y completes the construction.

The chart is read with the help of a thread stretched across the scales. It has the property that the values intercepted on the scales by any straight line are simultaneous values of the several variables. This is obvious, because, on such a chart, a straight line corresponds to a point on a Cartesian nomogram. Therefore the intercepts of the line on the scales correspond to the values of the variables represented by the scales at the point of the Cartesian nomogram which is the correlative of the straight line in question.

D'Ocagne's method has been widely applied and many expositions of the subject are now available. For further information his own treatise[109] or that of Lipka[110] may be consulted.

It is easy to see that this method is only applicable to Cartesian nomograms on which the contour lines are straight or may be so regarded without serious error. In the present case, however, the curvature of the contour lines of oxygen tension is considerable. Nevertheless, by confining our attention to the region of the chart where the values of pCO_2 fall within the physiological range, it is possible to replace the pO_2 contour curves by straight lines, without introducing serious error. We may then proceed to the transformation, which results in figure 40.

From this nomogram it is possible to read directly or to deduce by simple computation the magnitudes of all known phenomena of the respiratory function of blood. It should be clearly understood that figure 40 is a quantitative description of a particular specimen of blood taken from T.J.F. on March 23, 1927. For other specimens of human blood, for the blood of other species, and for the blood of pathological states, changes occur in the nomogram. Such changes, however, affect only the magnitudes and the minor peculiarities in the disposition of the scales. For example, the slope of the scale of r's is vari-

[109] M. d'Ocagne, *Traité de nomographie,* 2d ed., Paris, 1921.

[110] J. Lipka, *Graphical and Mechanical Computation,* New York, 1921.

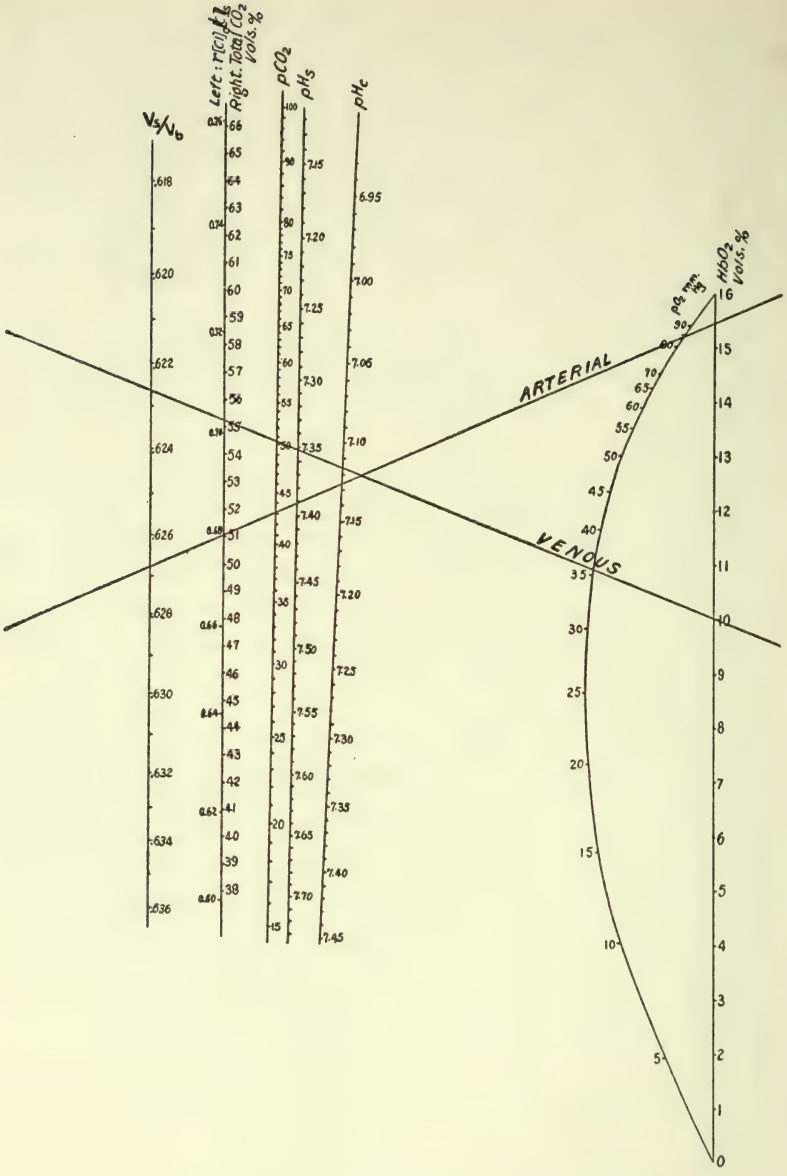


Fig. 40

Alignment Chart for Human Blood

able, but this may be due to the difficulty of making accurate estimates of small differences.

The conclusion seems to be justified that the general form of the nomogram, figure 40, represents the law of the blood which we have been seeking throughout the preceding chapters. With the consideration of differences from rest to work, from the normal to the pathological, and from species to species, we enter a field in which the comparative biological method becomes quantitative and rational.

On figure 40 seven variables are represented. A large number of others are, however, implicitly defined, and may be deduced by means of simple computations. In order to avoid the necessity of making such computations, it is sometimes more convenient to construct additional scales. This may be done as follows: Let u be a linear function of two of the variables for which scales exist, say $u = f(x, y)$. Choose any value of u, say u_1, and any convenient pair of values of x, say x_1 and x_2. Substitute in turn u_1 and x_1 and u_1 and x_2 in the equation $u = f(x, y)$. Solve the resulting two equations, thus obtaining the values y_1 and y_2. Then the intersection of the two straight lines joining x_1 with y_1 and x_2 and y_2 is the point corresponding to u_1. In like manner, the points u_2, u_3, . . . may be found and the scale of u constructed.

A number of such constructions have been performed and the results reproduced on the large nomogram, figure 41. This figure represents the blood of A.V.B. at rest and is chosen because it is based upon an exceptionally exhaustive study which affords a favorable opportunity for the methodical discussion of the system, considered as a whole.[111]

It will perhaps suffice to define the variables represented by the several scales of figure 41. Taken in order from left to right they are as follows:

I. $(Cl)_s$ The concentration of serum chloride, expressed in millimoles per liter of serum.

[111] Henderson, Bock, Field, and Stoddard, *Journal of Biological Chemistry*, LIX, 379 (1924).

II. *r*. Donnan's

$$r = \frac{[BHCO_3]_c}{[BHCO_3]_s} = \frac{[HCO_3^-]_c}{[HCO_3^-]_s} = \frac{[Cl^-]_c}{[Cl^-]_s} = \frac{[A^-]_c}{[A^-]_s} = \frac{[OH^-]_c}{[OH^-]_s} = \frac{[H^+]_s}{[H^+]_c}.$$

Here the brackets represent concentrations per liter of water of serum or cells, as the case may be.

III. $(Cl)_c$. The concentration of cell chloride, expressed as millimoles per liter of cells.

IV. a. $\Delta(Cl)$. The difference, expressed as millimoles per liter of blood, compared with arterial blood, in the total amount of chlorides in cells or serum.

IV. b. Per cent A in cells. The percentage of total blood chloride or bicarbonate present in the cells.

V. a. V of cells. The volume of the cells, expressed as percentage of this volume at about $O_2 = 80$ mm. and $CO_2 = 39$ mm., the point where this volume is 40 per cent of the total blood volume.

V. b. Per cent H_2O in cells. The percentage of total blood water present in the cells.

VI. BP_c. The base combined with cell protein, expressed as millimoles of base per liter of blood.

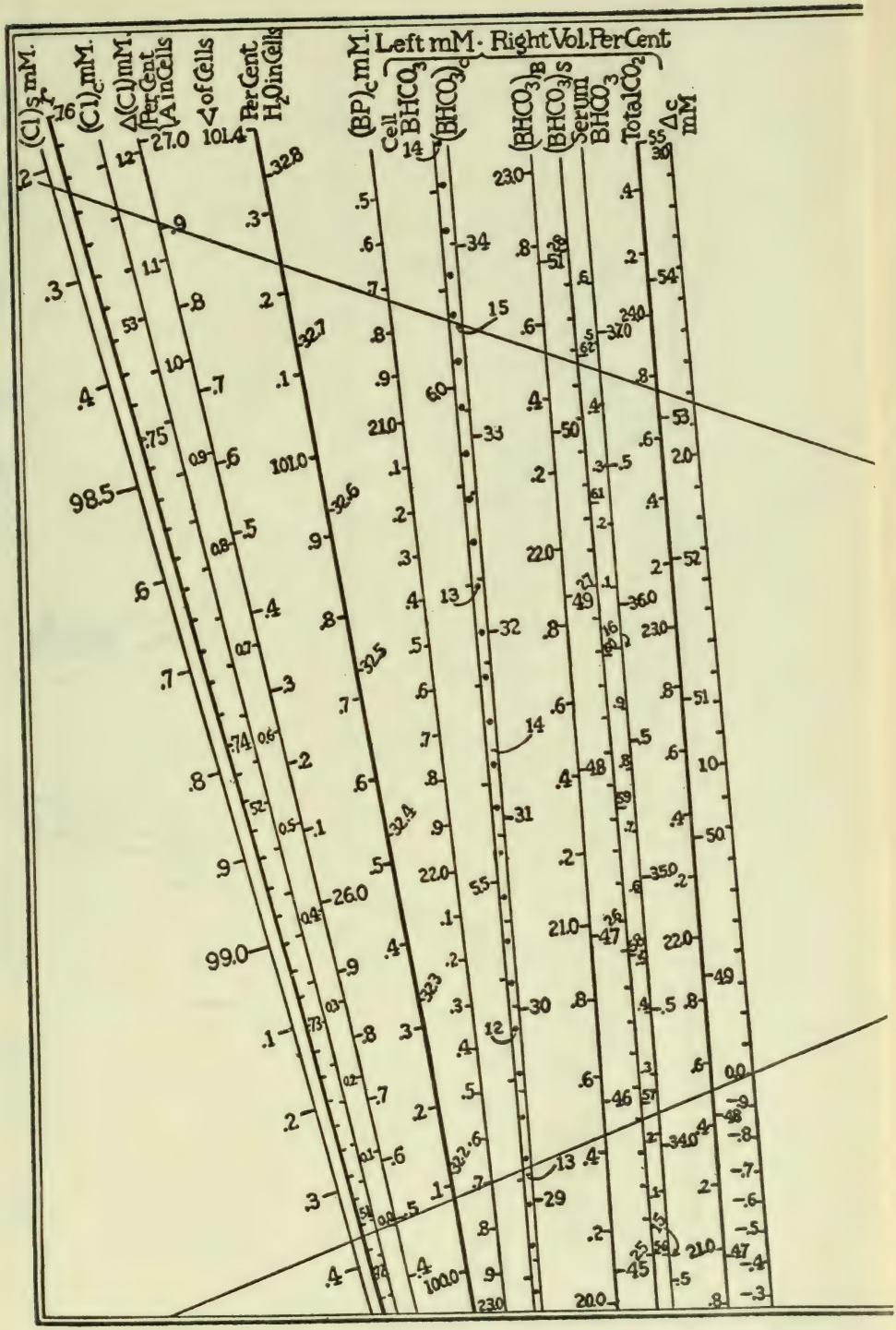
VII. Cell $BHCO_3$. The combined carbonic acid of the cells per liter of blood, expressed: (a) as millimoles, and (b) as volumes per cent.

VIII. $(BHCO_3)_c$. The combined carbonic acid of the cells per liter of cells, expressed: (a) as millimoles per liter, and (b) as volumes per cent.

IX. $(BHCO_3)_B$. The combined carbonic acid of whole blood, expressed: (a) as millimoles per liter, and (b) as volumes per cent.

X. $(BHCO_3)_s$. The combined carbonic acid of serum per liter of serum expressed: (a) as millimoles per liter, and (b) as volumes per cent.

XI. Serum $(BHCO_3)$. The combined carbonic acid of serum per liter of blood, expressed: (a) as millimoles per liter, and (b) as volumes per cent.


Left mM · Right Vol. Per Cent
$(Cl)_S$ mM.
$(Cl)_C$ mM.
$\Delta(Cl)$ mM.
Per Cent A in Cells
Per Cent H_2O in Cells
$(BP)_C$ mM.
Cell $BHCO_3$
$(HCO_3)_C$
$(BHCO_3)_B$
$(BHCO_3)_S$
Serum $BHCO_3$
Total CO_2
Δ_C mM
98.5
99.0
100.0
101.0
101.4
27.0
32.7
32.6
32.5
32.4
32.3
32.2
26.0

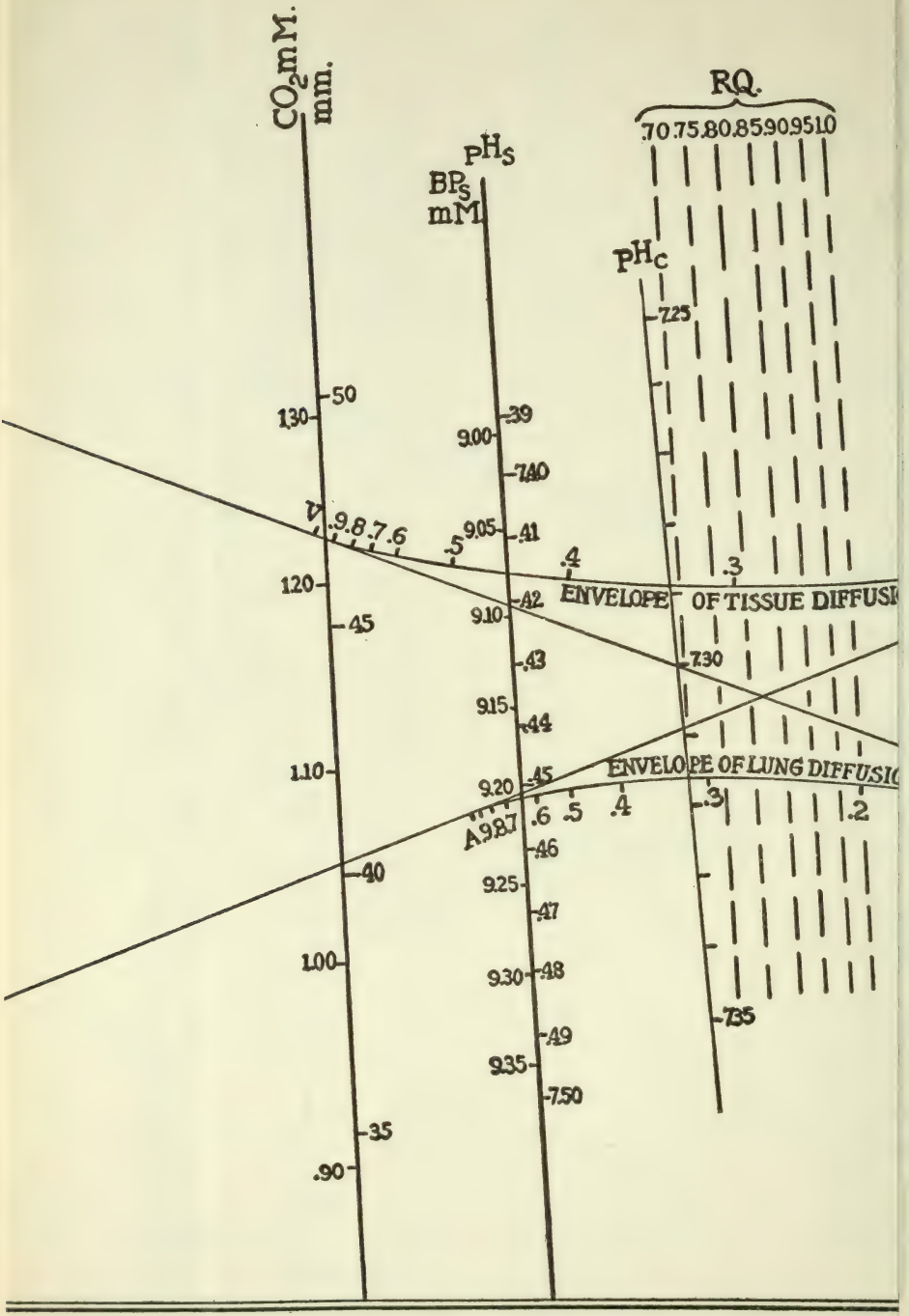


Fig. 41

Nomogram for the Blood of A.V.B.

XII. Total CO_2. The total carbonic acid of blood, expressed: (a) as millimoles per liter, and (b) as volumes per cent.

XIII. Δc. The change in concentration of total solute, compared with arterial blood, expressed in millimoles per liter of blood.

XIV. CO_2. Free carbonic acid, expressed: (a) as millimoles per liter of blood, and (b) as millimeters of partial pressure of mercury.

XV. a. BP_s. Base bound by protein of serum, expressed as millimoles of base per liter of blood.

XV. b. pH_s. $-\log [H^+]$ in serum.

XVI. pH_c. $-\log [H^+]$ in cells.

XVII. O_2. Oxygen pressure, expressed as millimeters of partial pressure of mercury.

XVIII. HbO_2. Combined oxygen, expressed: (a) as millimoles per liter of blood, and (b) as per cent of saturation. The scale for total oxygen is so nearly identical with this as to be practically indistinguishable from it.

Across the nomogram two straight lines are drawn. These lines define the equilibria of arterial blood and of venous blood, respectively.

The first is approximately true for all ordinary normal conditions; the second defines the conditions during moderate exercise. The graduated curves tangent to these lines near their point of intersection define the respiratory cycle. They will be discussed later.

The synthesis of our knowledge of the equilibria of the blood for the narrow physiological range of variations is completely represented by figure 41. We may now proceed to a systematic exposition of the different partial aspects of the phenomenon.

It has long been customary to represent two of these, the oxygen dissociation at different pressures of carbonic acid and the carbonic acid dissociation at different de-

grees of oxygenation, with the help of contour line charts. A third aspect is defined by the familiar equation,

$$[H^+] = k' \frac{[H_2CO_3]}{[BHCO_3]},$$

which is the analytical expression of a similar Cartesian nomogram.

The possibility of thus dealing with three variables at a time is nothing but the expression of the fact that, aside from variations in pO_2 and pCO_2, the blood is assumed to be subject to no change in composition, although in certain special cases this restriction is unnecessary. The fact that it is possible thus to define the variations of any three variables, independently of the other four, may readily be demonstrated as follows: Choose any three scales, say *u, v,* and *w,* on figure 40. Then, if values of any two of the three variables are given, *e.g.*, u_1, v_1, or u_2, w_2, or v_3, w_3, the third is determined. This is true because two points determine a straight line and the intersection of this line with the scale of the unknown variable determines the value, w_1 or v_2 or u_3, of this variable.

Now among 7 variables, taking 3 at a time, there are 35 combinations. Accordingly, the three cases above mentioned are but three among 35 cases necessary for an exhaustive description. Moreover, in each of these 35 cases three variables are involved. Accordingly, it is possible, in each case, to construct three contour line charts, taking in turn *u* and *v, u* and *w,* and *v* and *w* as the correlatives of *x* and *y,* the Cartesian coördinates. Thus a complete treatment involves the construction of 105 Cartesian contour line charts.

These 105 charts fall into 21 sets of five each. There are, in fact, 21 combinations, taking two at a time, among seven variables. Therefore, there are 21 pairs of Cartesian coördinates. When two of the variables have been chosen as Cartesian coördinates, five remain. Accordingly, they yield five families of contour lines. Evidently the five members of each of these 21 sets of contour line

charts form by superposition a Cartesian nomogram, which is a complete description of the system and the equivalent in all respects of figure 40. Figures 42 to 146 present these 105 charts arranged according to the plan of table 13.

TABLE 13.

Page	Figures	Cartesian coördinates	Contour lines
153	42-46	r, v	Total CO_2, CO_2 pressure, pH_s, HbO_2, O_2 pressure
154	47-51	r, total CO_2	v, CO_2 pressure, pH_s, HbO_2, O_2 pressure
155	52-56	r, CO_2 pressure	v, total CO_2, pH_s, HbO_2, O_2 pressure
156	57-61	r, pH_s	v, total CO_2, CO_2 pressure, HbO_2, O_2 pressure
157	62-66	r, HbO_2	v, total CO_2, CO_2 pressure, pH_s, O_2 pressure
158	67-71	r, O_2 pressure	v, total CO_2, CO_2 pressure, pH_s, HbO_2
159	72-76	v, total CO_2	r, CO_2 pressure, pH_s, HbO_2, O_2 pressure
160	77-81	v, CO_2 pressure	r, total CO_2, pH_s, HbO_2, O_2 pressure
161	82-86	v, pH_s	r, total CO_2, CO_2 pressure, HbO_2, O_2 pressure
162	87-91	v, HbO_2	r, total CO_2, CO_2 pressure, pH_s, O_2 pressure
163	92-96	v, O_2 pressure	r, total CO_2, CO_2 pressure, pH_s, HbO_2
164	97-101	Total CO_2, CO_2 pressure	r, v, pH_s, HbO_2, O_2 pressure
165	102-106	Total CO_2, pH_s	r, v, CO_2 pressure, HbO_2, O_2 pressure
166	107-111	Total CO_2, HbO_2	r, v, CO_2 pressure, pH_s, O_2 pressure
167	112-116	Total CO_2, O_2 pressure	r, v, CO_2 pressure, pH_s, HbO_2
168	117-121	CO_2 pressure, pH_s	r, v, total CO_2, HbO_2, O_2 pressure
169	122-126	CO_2 pressure, HbO_2	r, v, total CO_2, pH_s, O_2 pressure
170	127-131	CO_2 pressure, O_2 pressure	r, v, total CO_2, pH_s, HbO_2
171	132-136	pH_s, HbO_2	r, v, total CO_2, CO_2 pressure, O_2 pressure
172	137-141	pH_s, O_2 pressure	r, v, total CO_2, CO_2 pressure, HbO_2
173	142-146	HbO_2, O_2 pressure	r, v, total CO_2, CO_2 pressure, pH_s

The construction of any one of these charts may be explained as follows: Let the variables in question be x, y, and z, represented by the scales u, v, and w of figure 41, and let it be required to draw contour lines representing values of z on a Cartesian background of x and y. Choose suitable values for the z contour lines and find on w the points corresponding to these values. Through each of these points pass several straight lines and read the pairs of values of x and y defined by the intercepts of these lines on u and v. After tabulating the data thus obtained, it only remains to transfer them to a Cartesian background of x and y, and to join each set of points corresponding to each of the values of z.

On each of figures 42 to 146 the respiratory cycle and the arterial and venous points are represented. The cycle has been calculated in a manner which will be later explained.

The figures contain nothing that is not also contained in figure 40. But, like the two familiar charts, figures 100 and 145, which they include, they facilitate the understanding of the details of the respiratory process and systematically represent in turn all these details.

We have now completed the synthetic treatment of the physico-chemical system. The problem next arises of describing the operation of this system under physiological conditions.

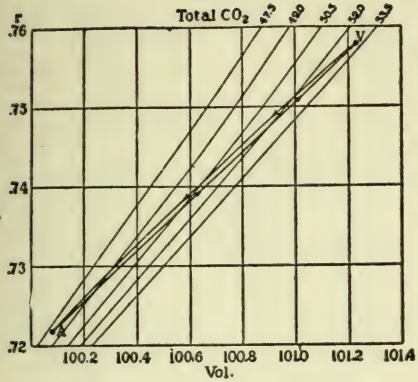


FIG. 42.

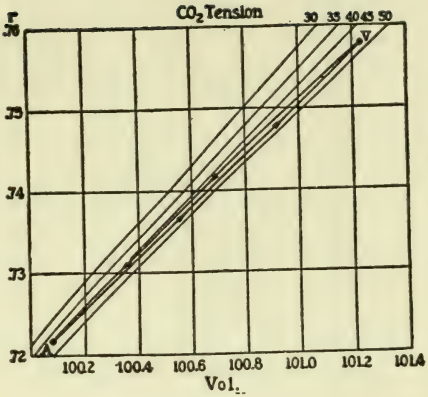


FIG. 43.

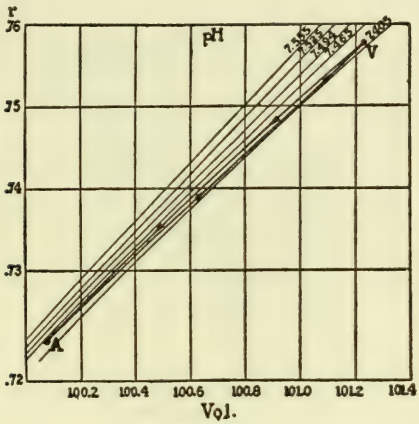
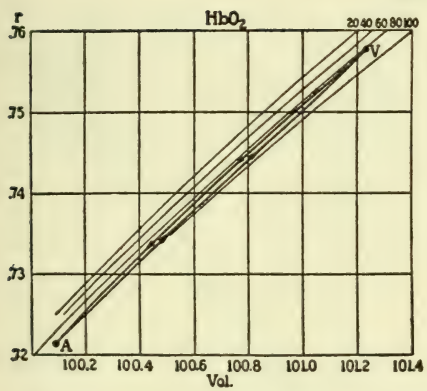


FIG. 44.



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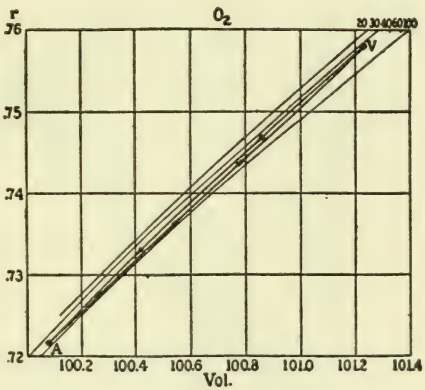


FIG. 46.

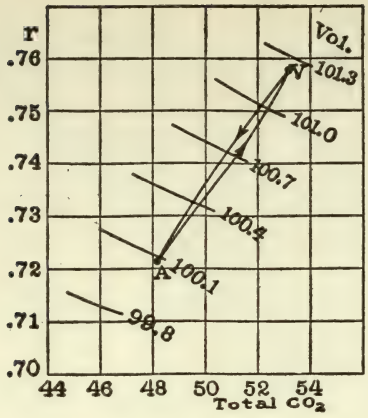


FIG. 47.

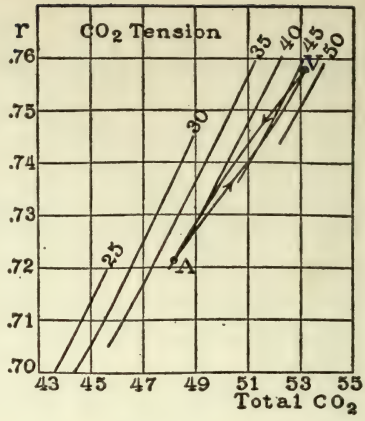


FIG. 48.

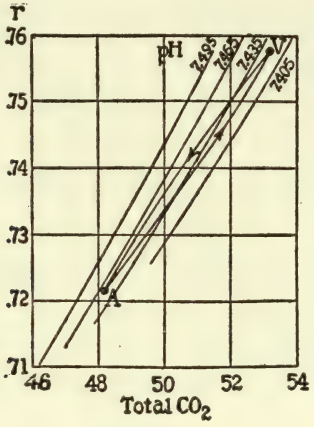
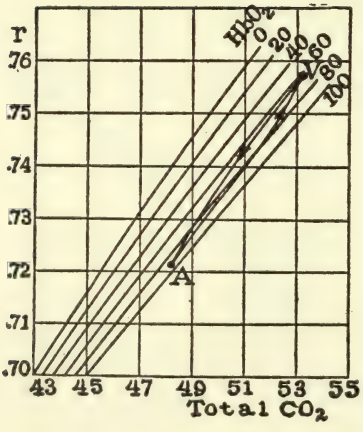


FIG. 49.



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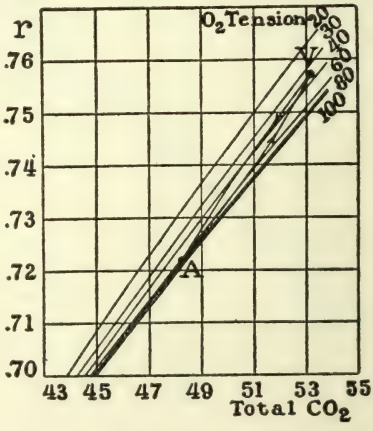


FIG. 51.

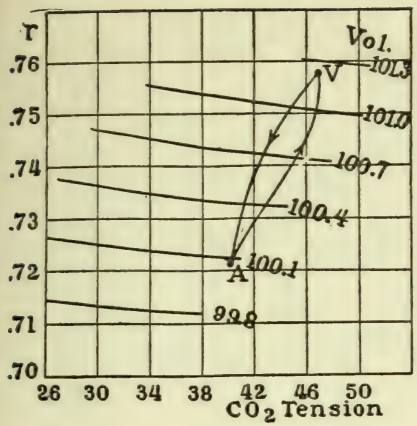


FIG. 52.

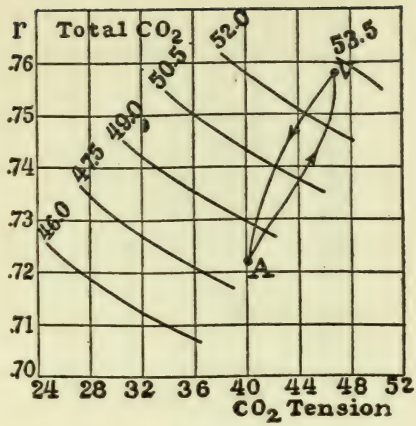


FIG. 53.

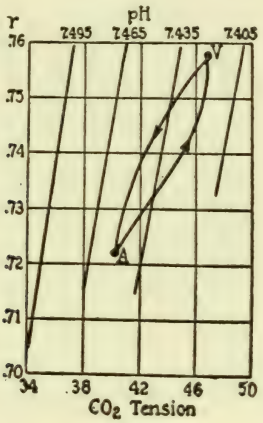


FIG. 54.

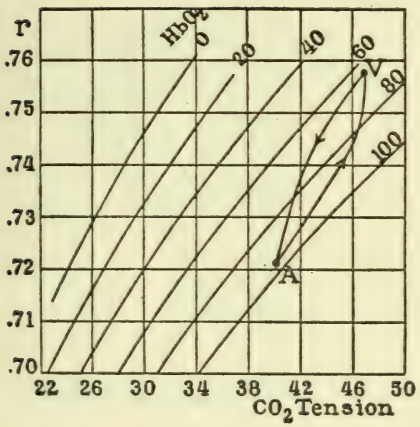


FIG. 55.

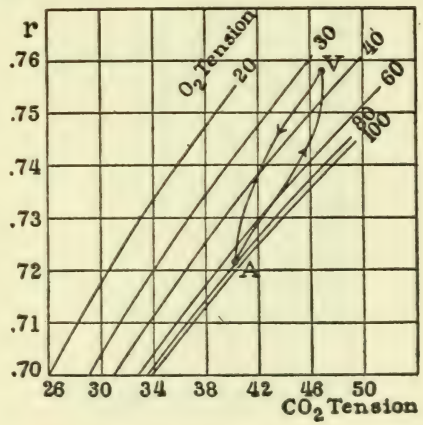
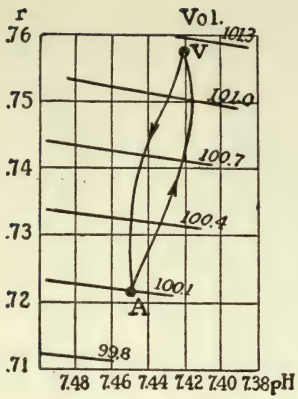


FIG. 56.



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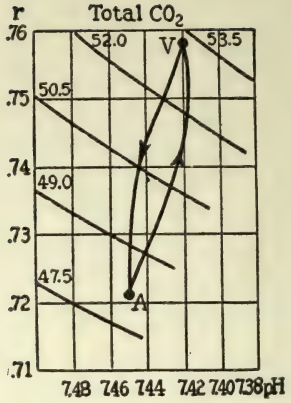


FIG. 58.

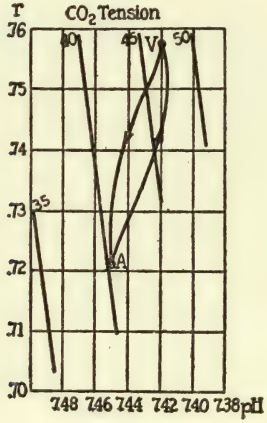
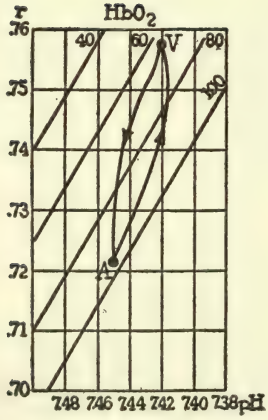


FIG. 59.



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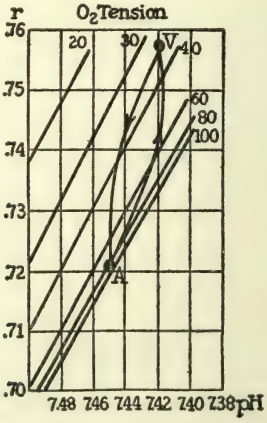


FIG. 61.

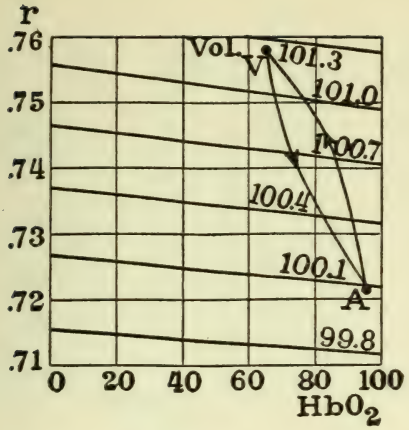


FIG. 62.

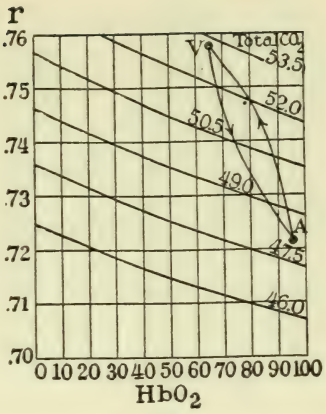


FIG. 63.

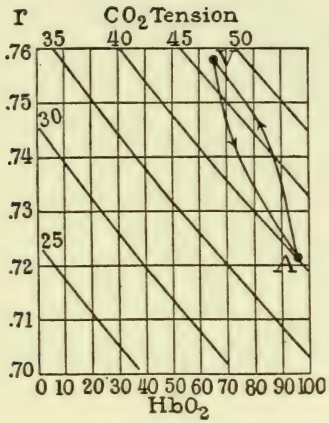


FIG. 64.

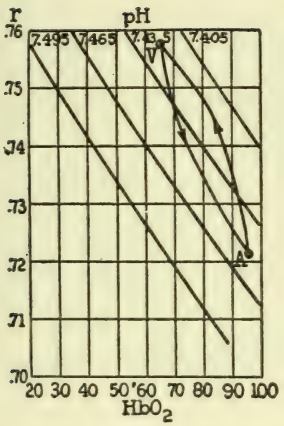


FIG. 65.

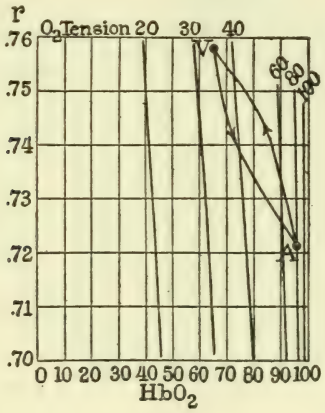


FIG. 66.

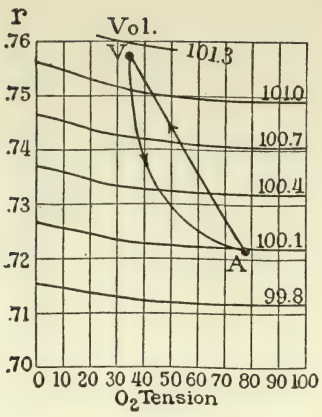


FIG. 67.

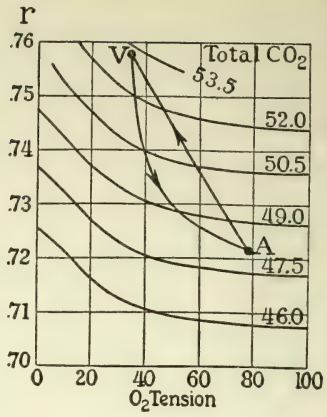


FIG. 68.

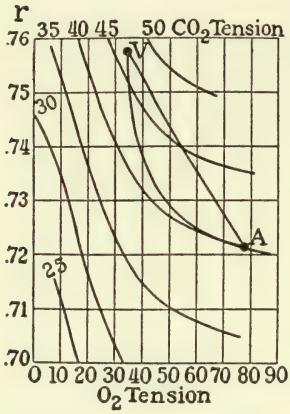


FIG. 69.

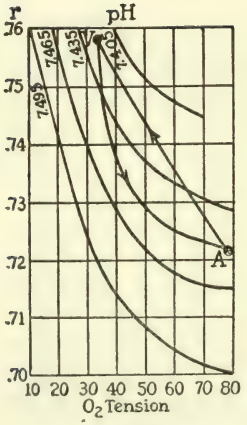


FIG. 70.

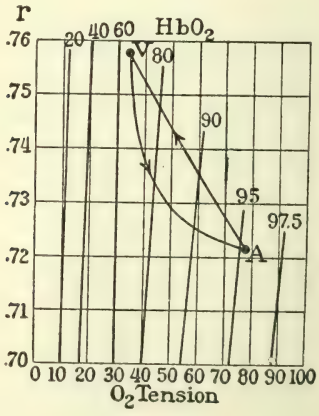


FIG. 71.

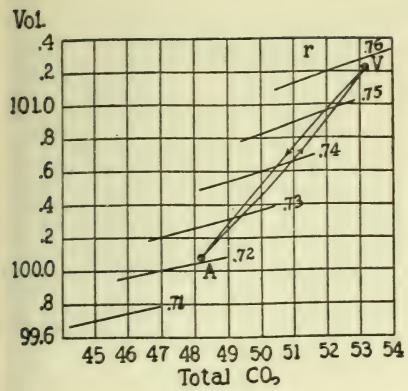


FIG. 72.

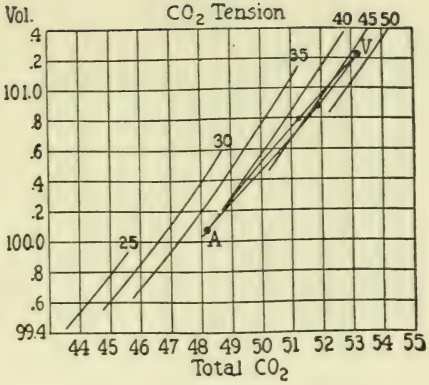


FIG. 73.

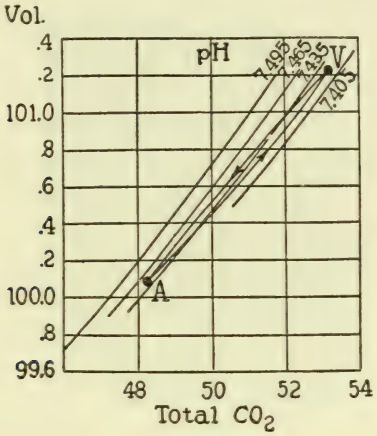


FIG. 74.

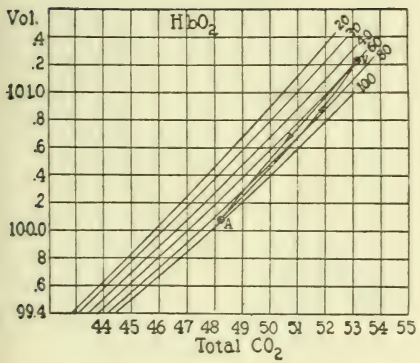


FIG. 75.

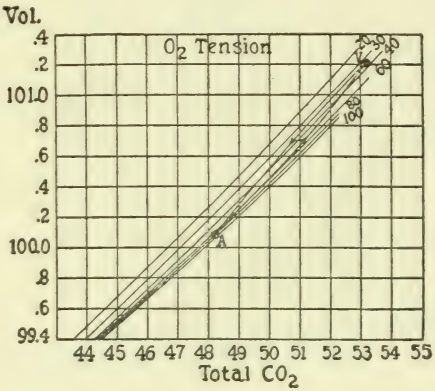


FIG. 76.

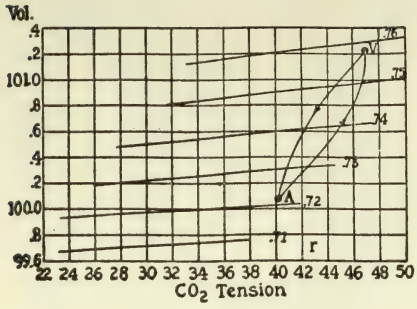


FIG. 77.

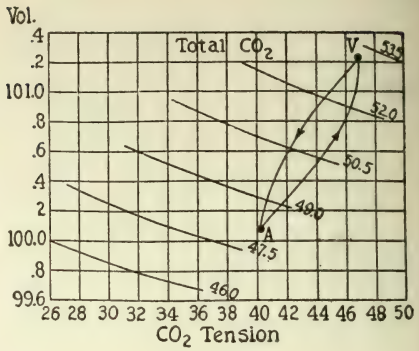


FIG. 78.

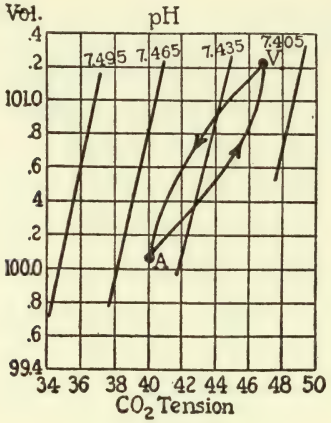


FIG. 79.

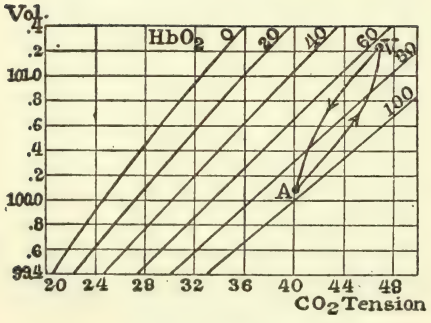


FIG. 80.

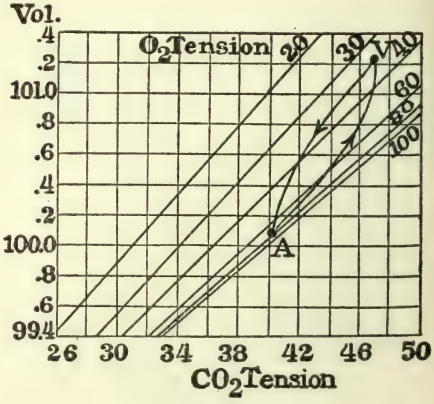


FIG. 81.

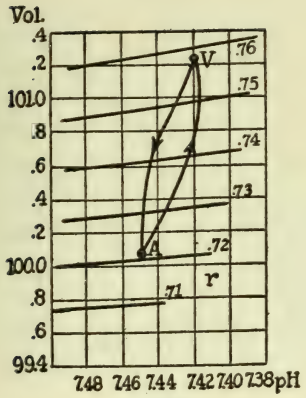


FIG. 82.

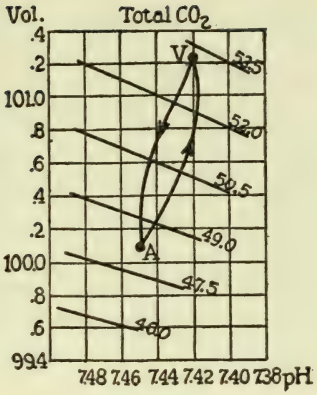


FIG. 83.

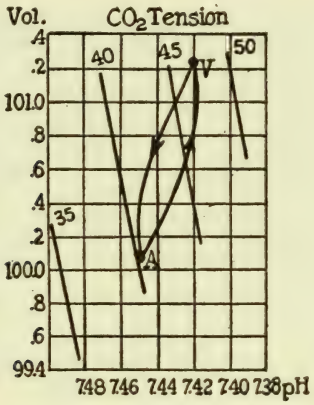


FIG. 84.

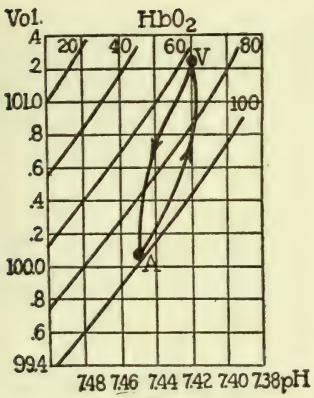


FIG. 85.

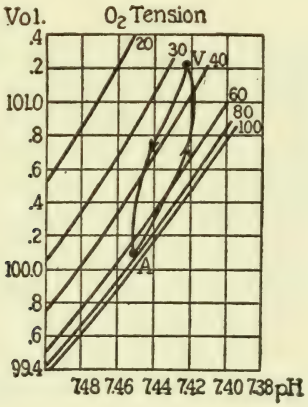


FIG. 86.

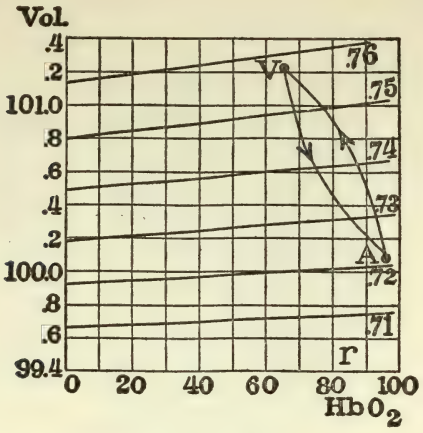


FIG. 87.

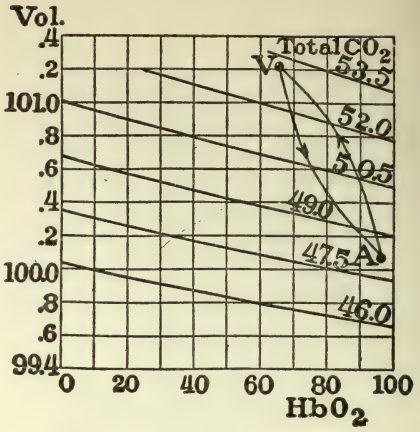


FIG. 88.

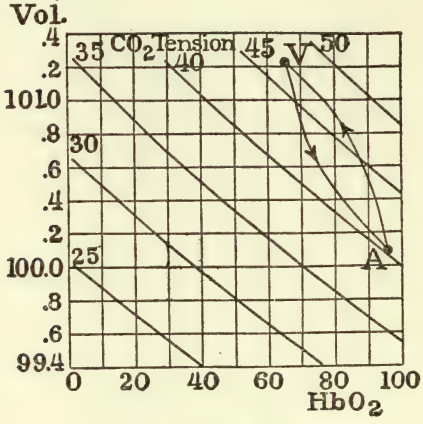


FIG. 89.

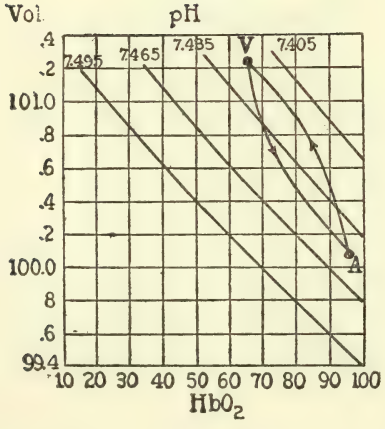


FIG. 90.

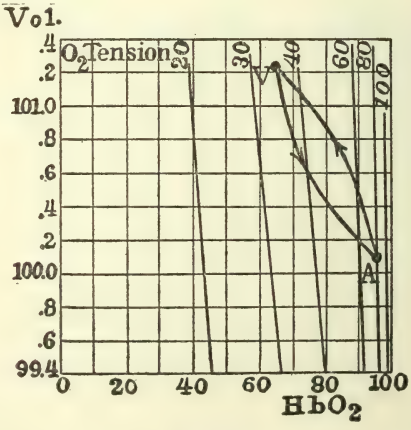


FIG. 91.

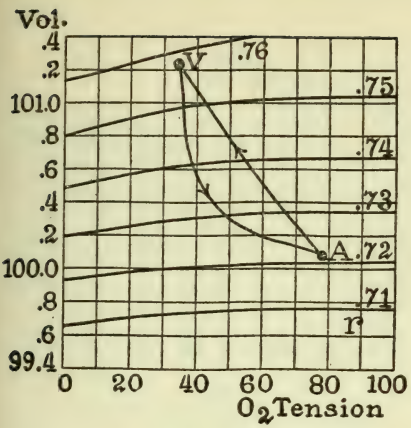


FIG. 92.

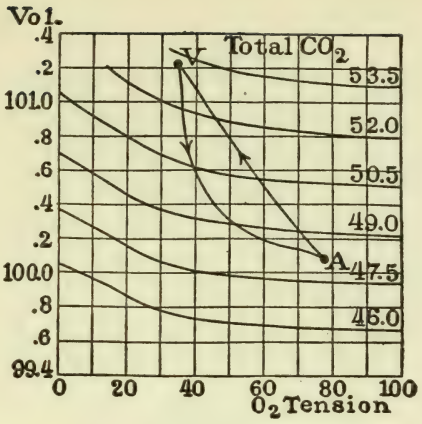


FIG. 93.

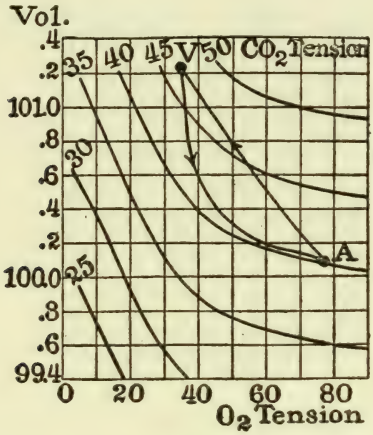


FIG. 94.

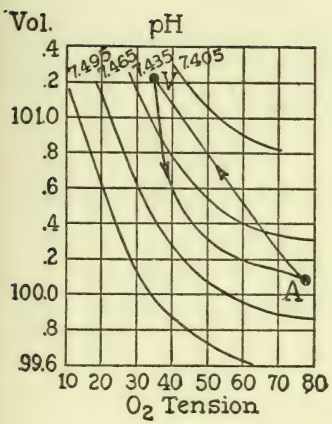


FIG. 95.

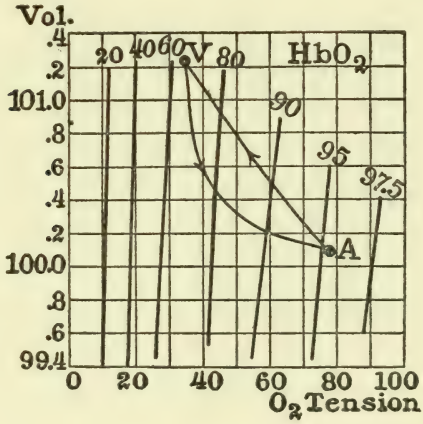


FIG. 96.

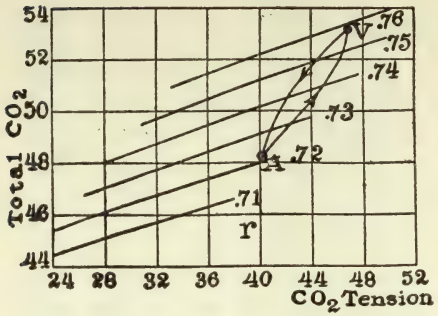


FIG. 97.

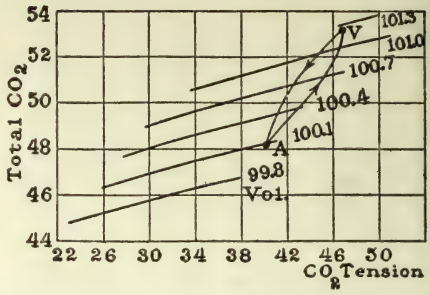


FIG. 98.

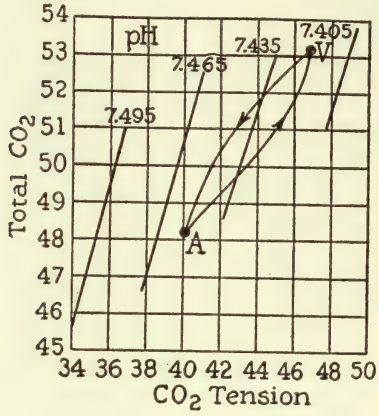


FIG 99.

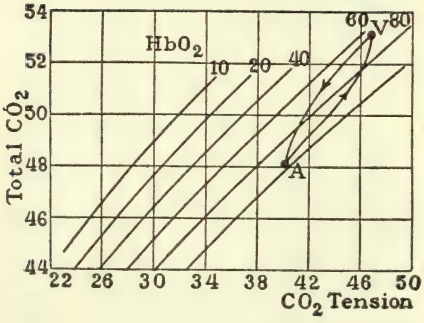
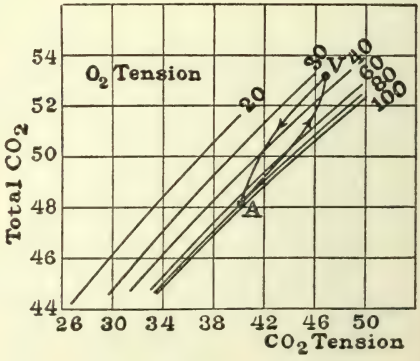


FIG. 100.



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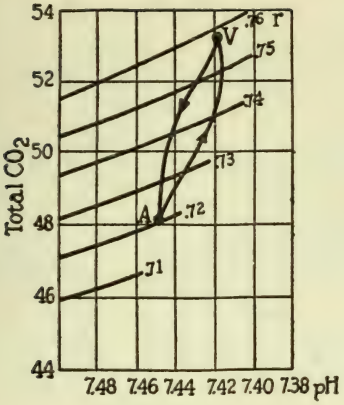


FIG. 102.

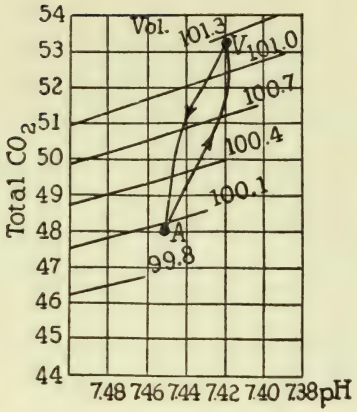


FIG 103.

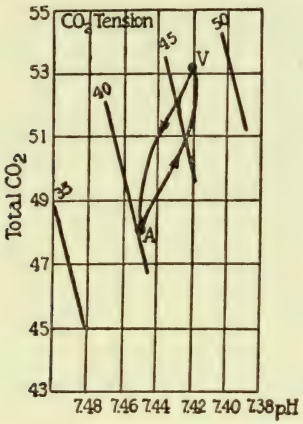


FIG. 104.

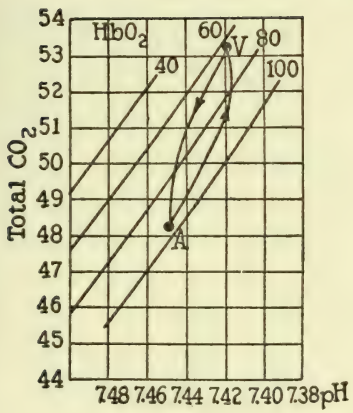


FIG. 105.

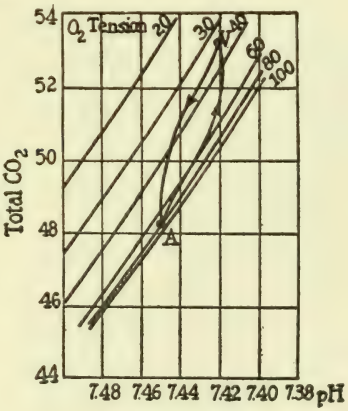


FIG 106.

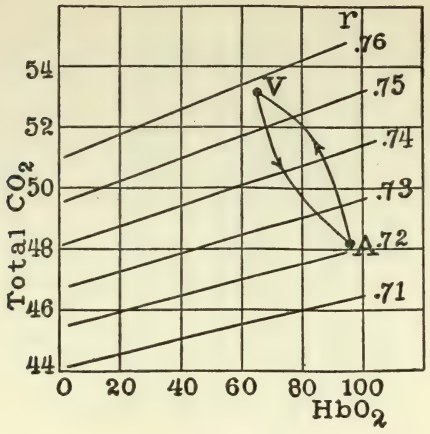
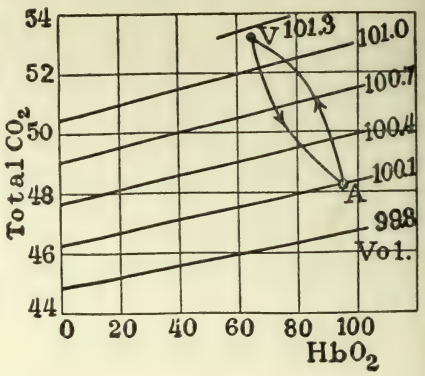


FIG. 107.



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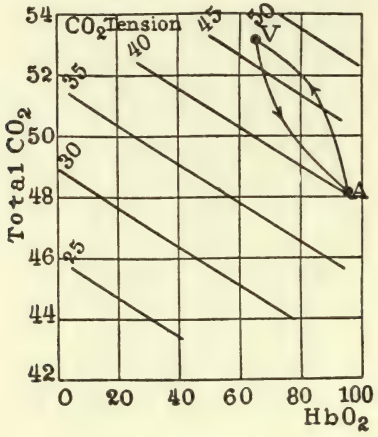

FIG. 109.

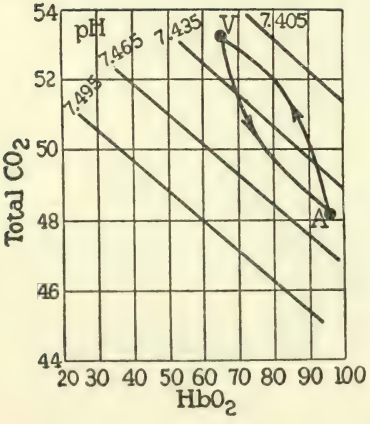

FIG. 110.

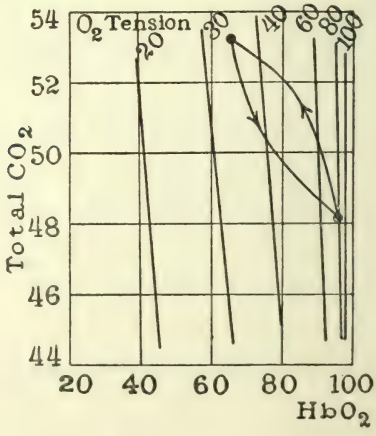

FIG. 111.

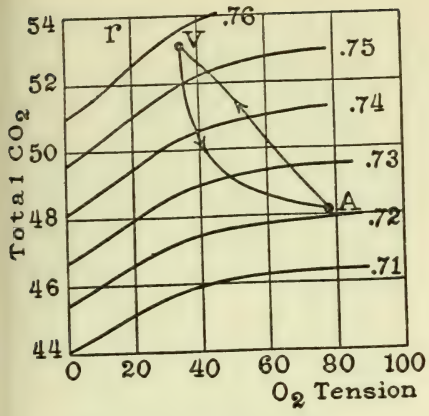

FIG. 112.

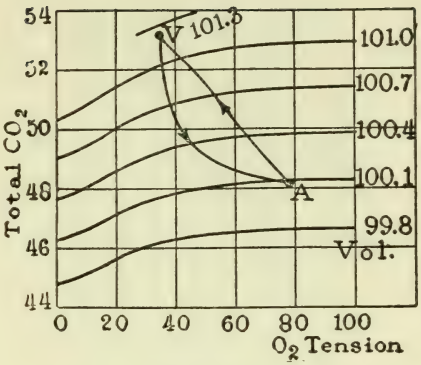

FIG. 113.

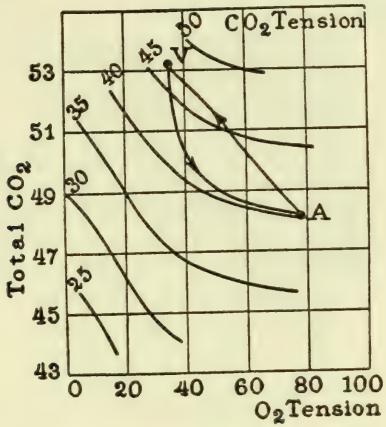

FIG. 114.

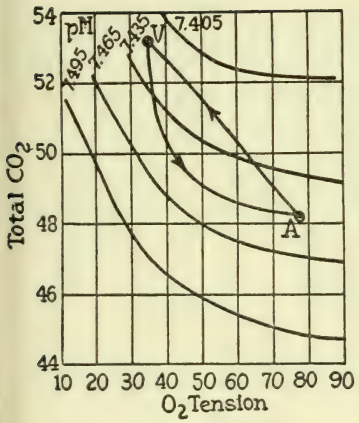

FIG. 115.

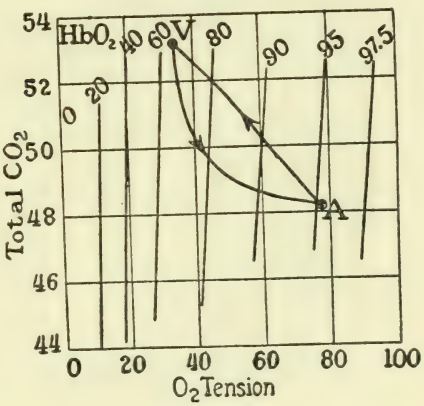

FIG. 116.

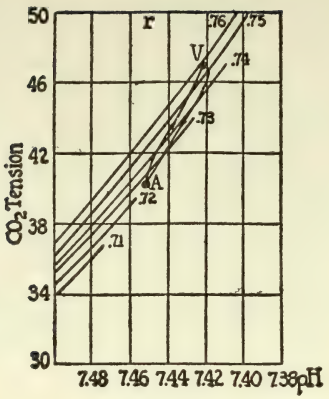

FIG. 117.

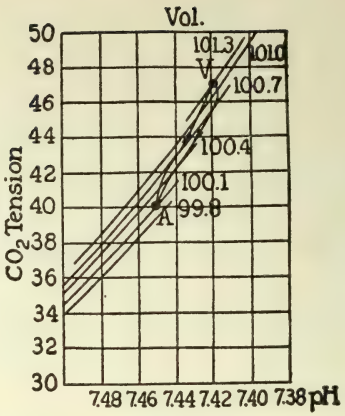

FIG. 118.

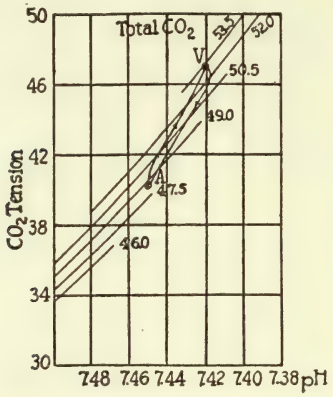

FIG. 119.

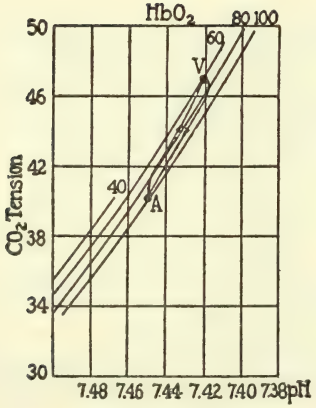

FIG. 120.

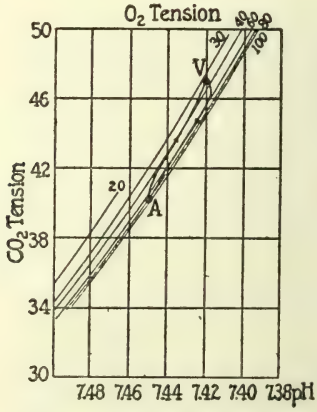

FIG. 121.

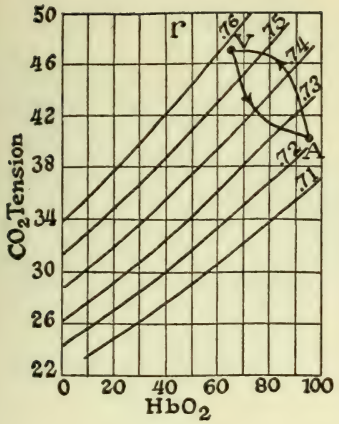

FIG. 122.

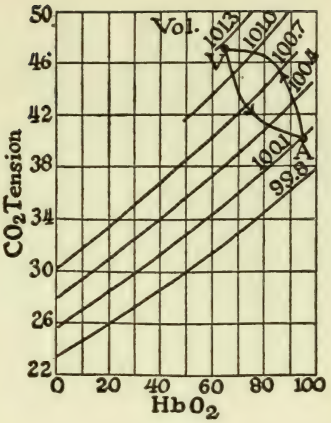

FIG. 123.

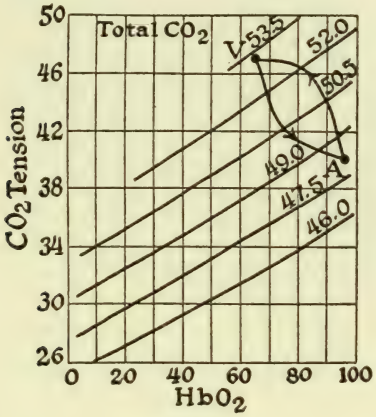

FIG. 124.

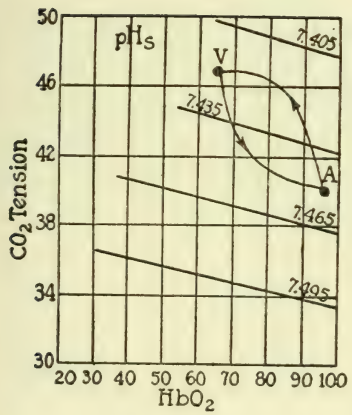

FIG. 125.

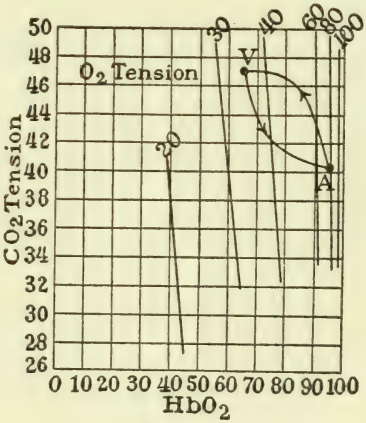

FIG. 126.

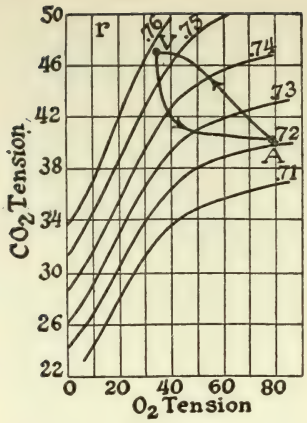

FIG 127.

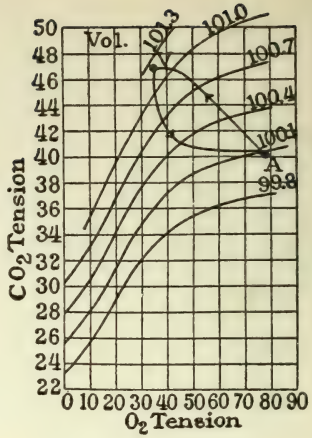

FIG. 128.

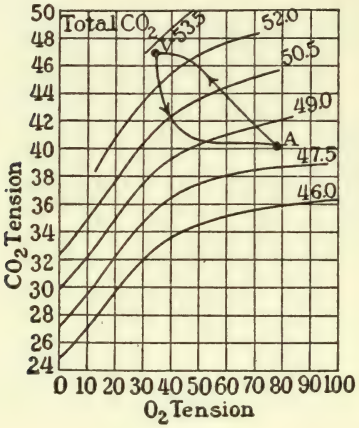

FIG. 129.

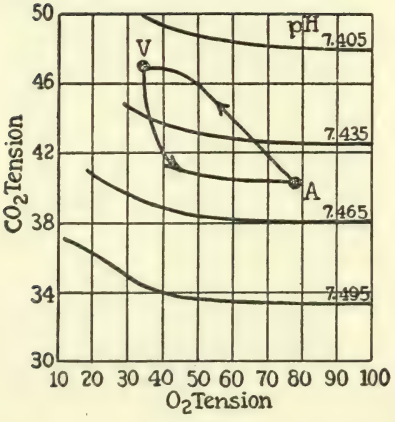

FIG. 130.

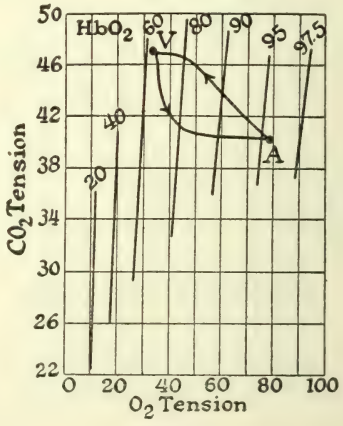

FIG. 131.

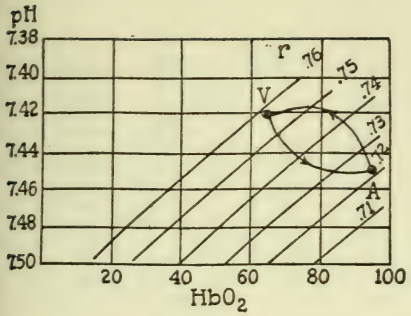

FIG. 132.

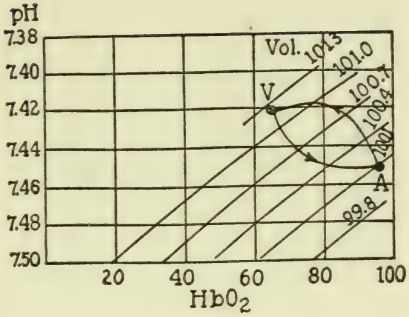

FIG. 133.

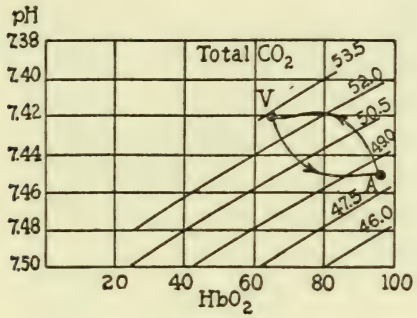

FIG. 134.

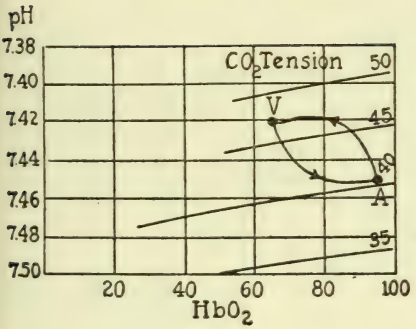

FIG. 135.

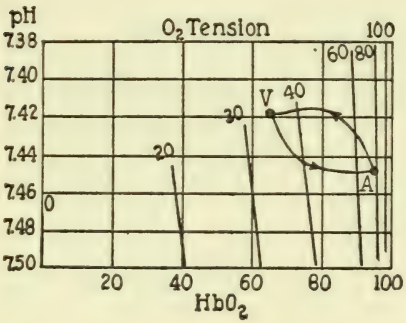

FIG. 136.

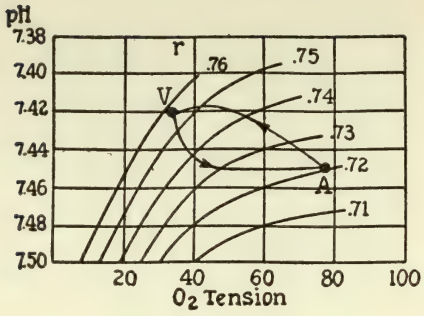

FIG. 137.

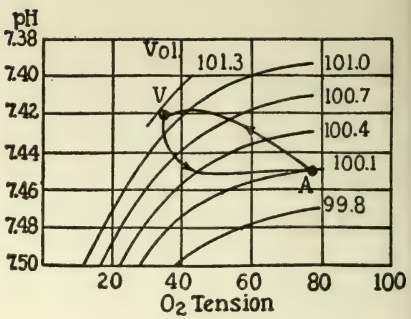

FIG. 138.

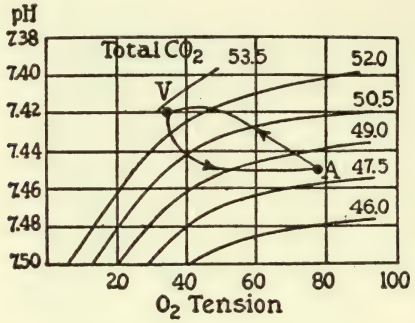

FIG. 139.

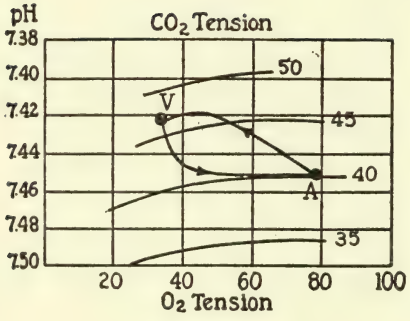

FIG. 140.

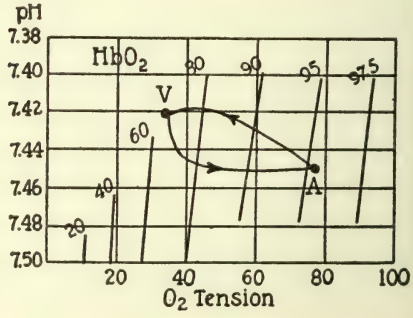

FIG. 141.

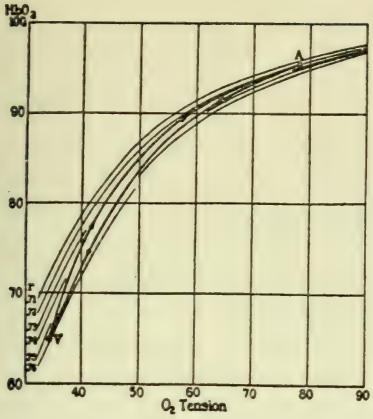

FIG. 142.

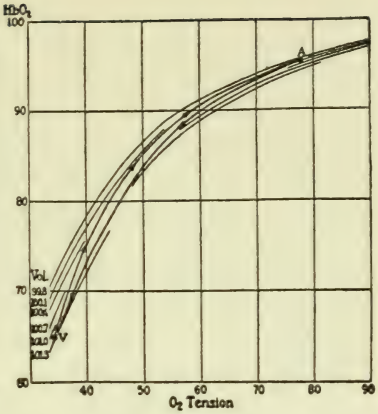

FIG. 143.

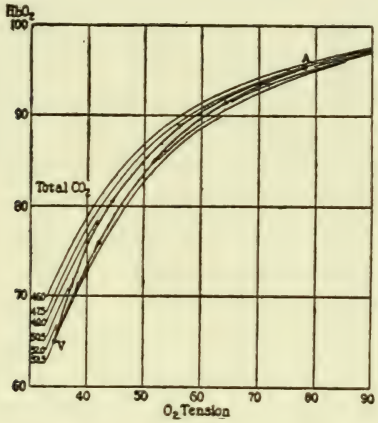

FIG. 144.

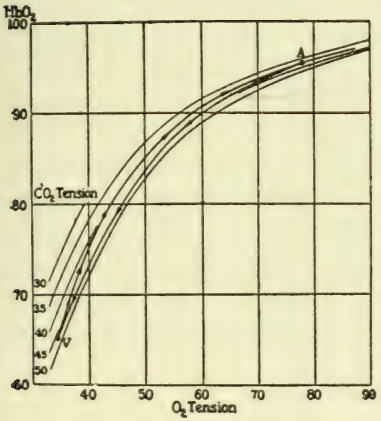

FIG. 145.

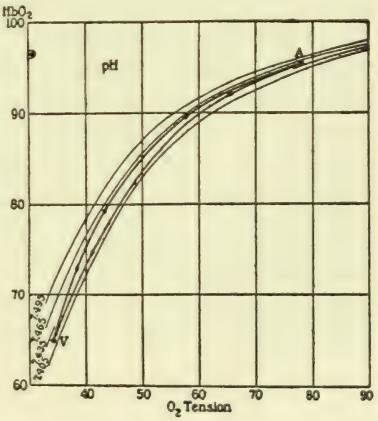

FIG. 146.

CHAPTER VII

THE RESPIRATORY CYCLE

WE have now at our disposal a quantitative description of the physico-chemical system that has engaged our attention in the preceding chapters. This description, though far from complete, and, no doubt, far from precise, completely fulfills the specifications stated in the second chapter. Therefore, on the one hand, it is conditioned by those approximations, omissions, and simplifications which we have deemed necessary in order to obtain a serviceable instrument for the study of the physiological activity of blood, and, on the other hand, if we have not been at fault, it embodies the facts and relations which are sufficient for that purpose. With the help of this description of the blood we shall now study the respiratory cycle.

On an alignment nomogram every straight line defines, by its intercepts of the several scales, simultaneous values of the corresponding variables. Therefore, on figure 40 every straight line defines for the blood of A.V.B. one state of equilibrium. Since the straight line is determined by any two points, it is sufficient, in order to define completely the composition of the arterial blood of A.V.B. to determine the values of two of the variables in a small sample of blood obtained by arterial puncture. Proceeding in this manner it has been found that the concentration of total carbonic acid is 21.5 millimoles per liter and that of combined oxygen 8.5 millimoles per liter. Accordingly, a straight line has been drawn on figure 41 through the points of the scales labeled Total CO_2 and HbO_2 that correspond to the values 21.5 millimoles and 8.5 milli-

moles respectively, and this line is labeled *Arterial Blood Line.*[112]

Next the position of the line that defines the composition of mixed venous blood must be determined. Concerning this, information may be deduced from the value of the respiratory quotient. In conformity with the method of construction of the nomogram, the scales of total carbonic acid and of oxyhemoglobin (approximately total

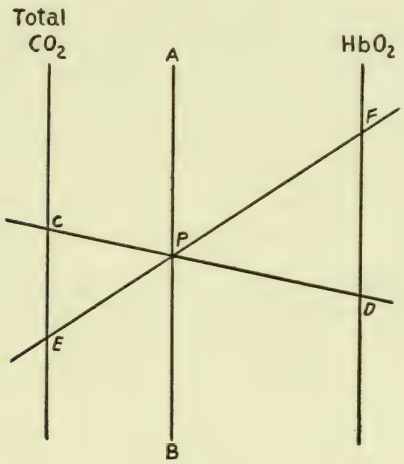

Fig. 147

Determination of Respiratory Quotient

oxygen) are parallel straight lines. In order to fix our ideas let a third line AB be drawn between these two scales and parallel with them. Through any point P of this line let two other lines CPD and EPF be so drawn as to intersect the scales of total CO_2 and HbO_2 and to enclose portions, CE and FD, of these scales between them. Figure 147 shows this construction. Then, whatever the angle between the lines CPD and EPF, we have from ele-

[112] Henderson, Bock, Field, and Stoddard, *Journal of Biological Chemistry,* LIX, 428 (1924).

mentary geometry $\frac{CE}{FD}$ = constant. But, if CPD be the venous blood line and EPF the arterial blood line, we have also, since the graduations of the scales of total CO_2 and of HbO_2 are both uniform, CE proportional to total carbonic acid excretion and FD approximately proportional to total oxygen consumption. Therefore the ratio $\frac{CE}{FD}$ determines the respiratory quotient, and any conditions for which the arterial blood line and the venous blood line intersect at any point on the line AB must be such that the respiratory quotient has a certain fixed value defined by the ratio $\frac{CE}{FD}$. If the scales of total CO_2 and of HbO_2 were graduated in the same unit of length we should have R.Q. $= \frac{CE}{FD}$. But in fact these units are different on figure 41, being about one-sixth greater for the scale of total CO_2 than for that of HbO_2 and therefore R.Q. $= \frac{6}{5} \times \frac{CE}{FD}$. Taking this disparity of the units of graduation into account the interrupted lines labeled R.Q. have been drawn on figure 41. Each of these lines is the locus of the points of intersection of arterial and venous blood lines for that value of the respiratory quotient which is printed above the line.

For A.V.B. under the conditions defined by the nomogram the respiratory quotient was found to have the value 0.82. Therefore the venous blood line must pass through the point of intersection of the arterial blood line with that line which is the locus of the points of intersection of arterial and venous blood lines for R.Q. = 0.82. This point is easily found by interpolation, between the lines marked R.Q. 0.80 and R.Q. 0.85, on the arterial blood line. When this point is known we require but one more point in order to determine the position of the venous blood line. In other words, we need quantitative informa-

tion concerning the concentration of but a single substance in the mixed venous blood. Unfortunately such information is not easy to obtain, for the mixed venous blood is inaccessible. A method has, however, been devised by Bock and Field,[113] and recently perfected by Bock and Dill,[114a] which enables us to estimate the partial pressure of carbon dioxide in the mixed venous blood. A description thereof will be found in the appendix. When the present nomogram was constructed, we were still unable to determine the position of even one more point on the venous blood line, and this line as drawn on the nomogram is hardly more than a guess. We now have reason to believe that it corresponds to a state of light muscular activity in which the consumption of oxygen is about 600 cc. per minute.

It must not be supposed that in the determination of the composition of the mixed venous blood this use of the R.Q. lines of the nomogram is anything but a convenient mathematical short cut. A moment's reflection will show that from (1) complete information concerning the composition of arterial blood, (2) the value of the respiratory quotient, and (3) the value of the concentration of any one substance in mixed venous blood, the values of the concentrations of other substances in mixed venous blood may be computed and the position of the venous blood line on the nomogram determined. The method above described is, however, perfectly convenient and very economical of labor. It is, therefore, to be preferred.

On figure 41 the line labeled R.Q. 0.70 approximately coincides with the scale of values of pH_c. From this relation the conclusion may be drawn that when the respiratory quotient of A.V.B. at rest has a value of about 0.70 there is no difference between the hydrogen ion concentration of the cells of arterial and of mixed venous bloods.

[113] Bock and Field, *Journal of Biological Chemistry,* LXII, 269 (1924); Field, Bock, Gildea, and Lathrop, *Journal of Clinical Investigation,* I, 65 (1924).

[114a] *Cf.* Appendix.

Under these circumstances the change in total carbonic acid of the respiratory cycle in blood must be due almost exclusively to the oxygen effect upon the affinity of hemoglobin for base, since at constant hydrogen ion concentration hemoglobin can exert no true buffer action, and with the small change in hydrogen ion concentration in serum the buffer action of the serum proteins is small.

TABLE 14.

	Individual		Mean	
A.V.B. Rest	(1)	0.70		
A.V.B. Rest	(2)	0.70		
A.V.B. Rest	(3)	0.71		
C.V.C. Rest		0.66		
A.V.B. Work		0.67	Normal Man	0.69
Pernicious Anemia	(1)	0.61		
Pernicious Anemia	(2)	0.73		
Pernicious Anemia	(3)	0.63	Pernicious Anemia	0.66
Polycythemia		0.59	Polycythemia	0.59
Myxedema	(1)	0.65		
Myxedema	(2)	[0.99]		
Myxedema	(3)	0.62	Myxedema	0.64
Horse (Van Slyke, Wu, and McLean)		0.75	Horse	0.75
Turtle 20°		0.57		
Turtle 37.5°		0.58	Turtle	0.58
			All Values	0.66

Another deduction from the same fact is that the effect upon the hydrogen ion concentration of the addition of one equivalent of oxygen is approximately equal to that of the addition of 0.7 equivalent of carbonic acid. This appears to be a roughly constant relation for different specimens of blood, as shown in table 14. In this table there will be found, for all cases thus far studied, the value of the respiratory quotient line which approximately corresponds with the scale of pH_c. The mean values are also indicated in the table, one datum from a case of myxedema, which was very imperfectly studied

so that the nomogram is of doubtful validity, being rejected. Otherwise, considering the great effect of small experimental errors upon the position of the pH_c scale, the approximation to constancy is very satisfactory. Since the blood of A.V.B. has been far more extensively studied than that of any other individual, we may regard the round number 0.7 as the most probable value of the respiratory quotient which corresponds to a difference of 0 between the values of pH_c in normal human arterial and venous bloods. It is certain that this value must vary slightly with variations in composition of the blood, it is probable that like most of our other estimates, it is affected with a small constant systematic error, but the general characteristics of the phenomenon are probably well defined.

When the arterial and venous blood lines have been located on the nomogram, the composition of arterial and of mixed venous bloods may be read directly from the intercepts of the scales. The values thus found and their differences, which measure the amplitude of the respiratory changes, are assembled in table 15. Tables 16 and 17 give corresponding values per unit of serum and cell volumes.

TABLE 15.

Blood.

Concentration of hemoglobin = 8.8 *mM* per liter blood.
Concentration of serum proteins = 51 gm. per liter blood.
Respiratory quotient = 0.82.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	549	260	809	544	265	809	−5	+5	0.0
B *mM* " " "	84.02	48.32	132.34	84.00	48.34	132.34	0.0	0.0	0.0
Cl " " " "	59.59	20.41	80.00	58.45	21.55	80.00	−1.13	+1.13	0.0
BP " " " "	9.20	22.70	31.90	9.09	20.73	29.82	−0.11	−1.97	−2.08
$BHCO_3$ " " " "	15.23	5.21	20.44	16.46	6.06	22.52	+1.23	+0.85	+2.08
H_2CO_3 " " " "	0.71	0.34	1.05	0.82	0.40	1.22	+0.11	+0.06	+0.17
Total CO_2 " " " "	15.94	5.55	21.50	17.28	6.46	23.75	+1.34	+0.91	+2.25
Free O_2 " " " "			0.07			0.03			−0.04
Combined O_2 " " " "		8.5	8.5		5.8	5.8		−2.7	−2.7
Total O_2 " " " "			8.6			5.8			−2.7
CO_2 tension, *mm. Hg.*			40			47			+7
O_2 tension " "			78			34			−44
Volume, *cc. per l. blood* ...	599.7	400.3	1,000	595.0	405.0	1,000	−4.7	+4.7	0.0
pH	7.450	7.309		7.421	7.300		−0.03	−0.01	
$r = \frac{[H^+]_s}{[H^+]_c} = \frac{[A^-]_c}{[A^-]_s}$			0.7215			0.7576			0.036
Per cent A in cells			25.49			26.90			1.4
Total concentration *mM per l.*									2.2

TABLE 16.

Serum.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	915.4	914.3	−1.0
B	*mM* " " "	140.1	141.1	+1.0
Cl	" " " "	99.37	98.22	−1.15
BP	" " " "	15.34	15.27	−0.07
$BHCO_3$	" " " "	25.40	27.66	+2.26
H_2CO_3	" " " "	1.17	1.38	+0.21
Total CO_2	" " " "	26.58	29.04	+2.46

TABLE 17.

Cells.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	649.5	654.2	+5
B	*mM* " " "	120.7	119.3	−1.4
Cl	" " " "	50.98	53.21	+2.23
BP	" " " "	56.70	51.18	−5.52
$BHCO_3$	" " " "	12.99	14.96	+1.97
H_2CO_3	" " " "	0.85	0.99	+0.14
Total CO_2	" " " "	13.86	15.95	+2.09
Combined O_2	" " " "	21.2	14.3	−6.9

In order to illustrate the use of the nomogram and of the tables we may seek in them a quantitative description of the exchanges between cells and serum which have been discussed at length in Chapter V. The last column of table 15 shows that the increase of blood bicarbonate in the passage through the capillaries of the greater circulation is 2.08 millimoles per liter and, in accordance with the approximations employed in constructing the nomogram, that the decrease in base bound by protein, BP, for whole blood is likewise 2.08 millimoles per liter. But reference to the two preceding columns shows that while in serum the increase in bicarbonate is 1.23 millimoles per liter blood, the decrease of BP is but 0.11 millimoles per liter blood. Further, in the cells the increase in bicarbonate is 0.85 millimoles per liter blood, but the decrease in BP is 1.97 millimoles per liter blood. Thus in

serum the increase of bicarbonate is 1.12 millimoles greater than the decrease in BP, in cells the increase of bicarbonate is 1.12 millimoles less than the decrease in BP. Meanwhile, however, 1.13 millimoles of chloride has moved from serum to cells, and the account balances. From these results we may draw the conclusion that hemoglobin is, in these circumstances, responsible for 95 per cent of the transport of carbonic acid, the serum proteins for but $\frac{11}{208}$ or five per cent. Of the variation of BP_c about 0.27 millimoles is due to true buffer action. Hence about $\frac{27}{208}$ or 13 per cent of the carbonic acid transported is dependent upon true buffer action of hemoglobin and 82 per cent is transported as a result of the oxygen effect. We shall return to this aspect of the process.

These exchanges may be summed up in the following equations, all of which may be verified with the help of table 15,

Arterial Blood BP — Venous Blood BP = Venous Blood $BHCO_3$ — Arterial Blood $BHCO_3$,

(Arterial Serum BP — Venous Serum BP) + (Arterial Serum Cl — Venous Serum Cl) = Venous Serum $BHCO_3$ — Arterial Serum $BHCO_3$,

Arterial Cell BP — Venous Cell BP = (Venous Cell $BHCO_3$ — Arterial Cell $BHCO_3$) + (Venous Cell Cl — Arterial Cell Cl).

There is no conclusive evidence that blood which has passed through different capillaries of the lung under normal conditions, differs widely in composition, and for many purposes we may perhaps regard the lung capillaries as approximately uniform diffusing surfaces, the blood issuing from them as approximately uniform arterial blood.[114b] In the greater circulation this is not the case, for there can be little doubt that conditions vary

[114b] But *cf.* Haldane, Meakins, and Priestley, *Journal of Physiology,* LII, 433 (1919).

widely from organ to organ and from tissue to tissue. During rest, for example, it is to be expected that venous blood from capillaries of the heart muscle may be significantly different from venous blood from capillaries in the biceps, the brain, or the kidney. Therefore the line labeled *Arterial Blood Line* on figure 41 probably gives a fair characterization of arterial blood, or at least of an average blood which is made up by mixing a multitude of parts not too different from one another to defeat our efforts to make use of this description. On the other hand, it is to be feared that estimates of the composition of mixed venous blood must be used with great caution, since the composition of this blood is the average obtained by mixing the many different local venous bloods from different parts of the body, and we can only form rough conjectures concerning the statistical properties of this class of objects. Especially it is to be feared that deviations from the mean may be distributed in such a manner as to render the solution of certain physiological problems very difficult, if not illusory. Be this as it may, we can but press on, remembering meanwhile that such obstacles are by no means peculiar to physiology and that in the long run they may be circumvented. One thing, at least, is certain: on entering the capillaries of the greater or of the lesser circulation, the blood, whether venous or arterial, is uniform, for it is thoroughly mixed in the heart and in the heart's efferent vessels.

The important observations of Peters[115] have shown that between arterial blood and venous blood significant variations in the masses of other components than oxygen and carbon dioxide may sometimes occur. This fact is to be ascribed to the formation of lymph, and promises to yield valuable results in the study of that process. We have not failed to examine the phenomenon, which is often small enough to be without effect upon our conclusions, and by the exercise of due caution we have been able to

[115] Peters, Bulger, and Eisenman, *Journal of Biological Chemistry*, LXVII, 165 (1926).

eliminate it from our considerations. The facts that we have observed are collected in table 18. When such

TABLE 18.

Total CO_2 of arterial and of venous blood.

A. RESTING SUBJECTS.

Subject	Date	Remarks	Total CO_2 at $pCO_2 = 40$ mm. Arterial	Venous	Δ Total CO_2
	1926		*vol. per cent*	*vol. per cent*	*vol. per cent*
J.S.L.	Feb. 12	Clinician	49.3	48.4	+0.9
	1927				
McDonald	Jan. 4	Leukemia	40.0	39.2	+0.8
A.H.	Jan. 11	Myxedema	51.0	49.2	+1.8
E.McD.	Jan. 15	Myxedema	48.6	49.3	−0.7
Tracy	Jan. 22	Nephritis	22.1	22.3	−0.2
J.F.	Jan. 28	Pernicious Anemia	60.7	59.1	+1.6
J.F.	Mar. 1	Pernicious Anemia	53.9	53.8	+0.1
J.F.	Mar. 23	Pernicious Anemia	50.1	49.8	+0.3
Bu	Feb. 14	Myxedema	39.3	38.5	+0.8
J.C.	Feb. 23	Student	48.8	47.6	+1.2
Bo	Sept. 9	Diabetic Coma	16.5	16.5	0.0
P.J.	Oct. 21	Hypertension	46.5	45.8	+0.7
J.H.T.	Nov. 16	Student	46.9	46.4	+0.5
Ro	Nov. 22	Nephritis	31.7	31.7	0.0
C	Nov. 27	Nephritis	25.1	26.8	−1.7
Ri	Dec. 9	Nephritis	27.9	30.2	−2.3

B. EXERCISING SUBJECTS.

Subject	Date	Oxygen used	Total CO_2 at $pCO_2 = 40$ mm. Arterial	Venous	Δ Total CO_2
	1926	*cc. per min.*	*vol. per cent*	*vol. per cent*	*vol. per cent*
A.V.B.	Jan. 19	1200	47.3	47.7	−0.4
A.V.B.	Jan. 26	1800	36.9	37.9	−1.0
A.V.B.	Feb. 3	1500	39.9	41.2	−1.3
A.V.B.	Feb. 17	1770	40.0	40.8	−0.8
L.M.H.	Jan. 14	1880	42.2	42.7	−0.5
L.M.H.	Feb. 9	1720	43.0	43.5	−0.5
D.B.D.	Feb. 11	1730	43.8	44.3	−0.5

changes are small enough to be neglected, figure 41 may be used to define the respiratory exchanges in any part

of the body for which the composition of the local venous blood is known. It may likewise be employed for the study of any hypothetical states of the respiratory cycle and as a means of illustrating many other peculiarities of the blood, in addition to those of the heterogeneous equilibrium which have just been discussed.

While figure 41 is the most convenient instrument for such purposes it should be remembered that, aside from uncertainties regarding the values of r for the different ions, figure 40, with its seven scales, includes all the necessary information for a complete description of the blood. Given these seven scales, all the others may be deduced by computation, with the aid of the definitions of the variables which they represent.

Now that we are in possession of a description of the properties of arterial blood and of mixed venous blood, we may undertake an examination of the process by which venous blood becomes arterial and arterial blood venous.[116]

In figure 148 the abscissas are values of total oxygen, the ordinates, values of total carbon dioxide, while values of oxygen pressure and carbon dioxide pressure appear as contour lines. Therefore the figure is nothing but a large-scale drawing of a portion of figure 33. Three points are marked on the figure: L, corresponding to the oxygen and carbon dioxide partial pressures of alveolar air, as directly determined; A, the arterial blood point, determined by repeated analyses of blood drawn from the radial artery; and V, the venous blood point. The position of this point must fall somewhere on the line marked R.Q. = 0.81, since this was the value of A.V.B.'s respiratory quotient for the conditions now under consideration, and it is evident that on this diagram any straight line drawn through the arterial point is a respiratory quotient line. The slope of the line is the measure of the respira-

[116] Henderson, Bock, Field, and Stoddard, *Journal of Biological Chemistry,* LIX, 424-430 (1924).

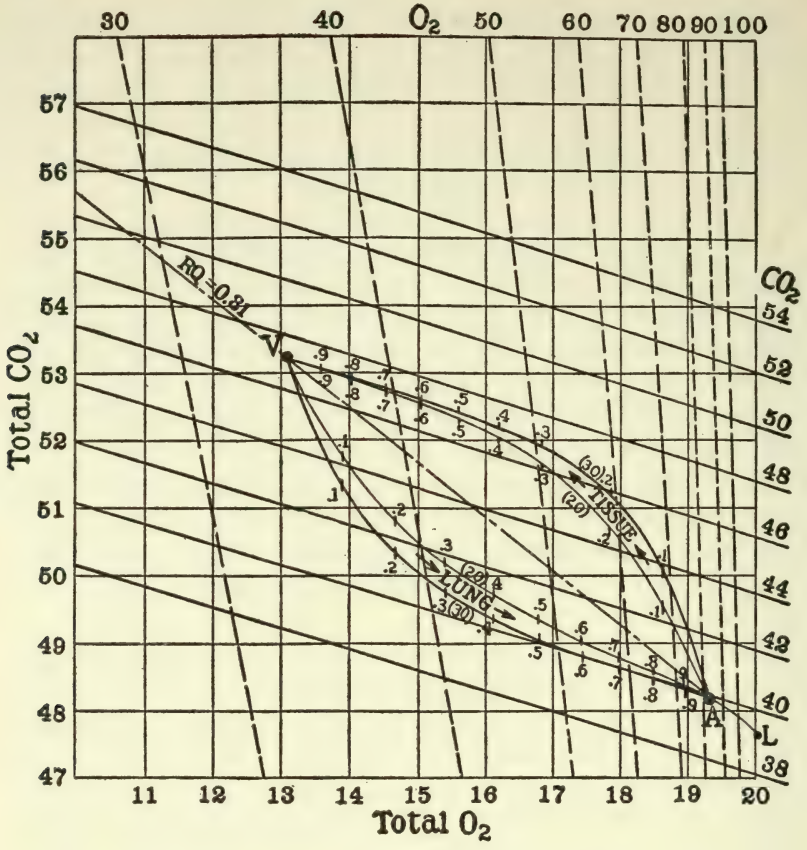

Fig. 148

The Respiratory Cycle

tory quotient. Therefore it is again necessary to know but one fact regarding the mixed venous blood in order to determine the position of the venous point on the diagram. Taking 47 mm. as the value of pCO_2 characteristic of mixed venous blood, the point is found on the R.Q. = 0.81 line by interpolation between the two contour lines for $pCO_2 = 46$ mm. and $pCO_2 = 48$ mm.

With the Cartesian coördinates, the pO_2 and pCO_2 con-

tour lines, and the points L, A, and V, once established, it is possible to take another step.

The rate of increase of the oxygen content of an infinitesimal portion of a capillary column of blood in the lungs must be proportional to the difference between the alveolar oxygen pressure $(pO_2)_L$, and that of the blood, $(pO_2)_B$:

$$\frac{d\,(\text{total } O_2)}{dt} = a_1\{(pO_2)_L - (pO_2)_B\} = a_1\Delta\,(pO_2).$$

Similarly,

$$\frac{d\,(\text{total } CO_2)}{dt} = a_2\{(pCO_2)_L - (pCO_2)_B\} = a_2\Delta\,(pCO_2).$$

Dividing, $$\frac{d\,(\text{total } CO_2)}{d\,(\text{total } O_2)} = \frac{a_2}{a_1} \times \frac{\Delta\,(pCO_2)}{\Delta\,(pO_2)}.$$

The value of the constant term $\frac{a_2}{a_1}$ is not accurately known. For water its value is about 20, for the tissues about 30.[117] But we are here concerned with conditions which are hard to define, since, to mention only one complication, the amount of mixing within the capillary, and hence the extent to which the exchanges between red cells and plasma are adjusted, remain unknown. We shall therefore make no attempt to estimate the value of $\frac{a_2}{a_1}$, but employ in turn the round values of 20 and 30 in order to discover, if possible, the general characteristics of the diffusion process.

For figure 148

$$\frac{d\,(\text{total } CO_2)}{d\,(\text{total } O_2)} = \frac{dy}{dx}.$$

Therefore it is evident that all points such that

$$\frac{a_2}{a_1} \times \frac{\Delta\,(pCO_2)}{\Delta\,(pO_2)} = m = \text{a negative constant},$$

[117] A. Krogh, Silliman Lectures, Lecture IX, *The Anatomy and Physiology of Capillaries,* New Haven, 1922.

or, in words, such that the difference between the carbon dioxide pressure at the point and the carbon dioxide pressure of the alveolar air, divided by the difference between the oxygen pressure at the point and the oxygen pressure of alveolar air is constant, are points which define a slope on the Cartesian coördinates.

The meaning of this slope may be understood from the following considerations. Instead of speaking of a point on the chart as defining a given condition of the blood, we may speak of the blood as existing at a point on the chart. Then, in order to reach the arterial point, the blood may be said to describe a curve upon the chart, and the direction in which the blood must be moving when at the point p is that of the slope m_p, in question. In other words, this slope is the slope of the tangent, at the point p, to the curve over which the blood must pass as a result of a diffusion process. This is true because, as already explained, when the blood is at the point p,

$$\frac{dy}{dx} = \frac{d\,(\text{total } CO_2)}{d\,(\text{total } O_2)} = \frac{a_2}{a_1} \times \frac{(pCO_2)_L - (pCO_2)_B}{(pO_2)_L - (pO_2)_B} = \frac{a_2}{a_1} \times \frac{\Delta\,(pCO_2)}{\Delta\,(pO_2)} = m_p.$$

It is, accordingly, convenient to draw a family of contour lines, each one such that for every point of the contour line

$$\frac{a_2}{a_1} \times \frac{(pCO_2)_L - (pCO_2)_B}{(pO_2)_L - (pO_2)_B} = \frac{a_2}{a_1} \times \frac{\Delta\,(pCO_2)}{\Delta\,(pO_2)} = m_p = \text{a constant.}$$

Here $(pCO_2)_B$ and $(pO_2)_B$ are the carbon dioxide pressure and oxygen pressure corresponding to any point, p, of the contour line and $(pCO_2)_L$ and $(pO_2)_L$ the carbon dioxide pressure and the oxygen pressure corresponding to the alveolar air point, L.

Next, taking $\frac{a_2}{a_1} = 20$, the characteristic slope, $\frac{dy}{dx} = \frac{d\,(\text{total } CO_2)}{d\,(\text{total } O_2)}$, defined by each contour line, is calculated, and a large number of short parallel lines of the calculated slope, each intersecting the contour line, are drawn.

It now remains to join the point V and the point L by means of a curve which cuts each of these contour lines so that the tangent to the curve at each point of intersection with a contour line is parallel with the characteristic slope defined by the contour line. The curve thus constructed is the required representation of the diffusion process in the lung.

Taking $\frac{a_2}{a_1} = 30$, a similar curve is obtained. These two curves are represented on figure 148 and are marked "LUNG (20)" and "LUNG (30)."

The analogous curves for the tissue diffusion process, assuming a condition in which the local venous blood is of the same composition as the mixed venous blood, are somewhat more difficult to obtain and also more uncertain. This depends upon the fact that, in the absence of information concerning oxygen and carbon dioxide pressures within the tissues, it is necessary to proceed by a method of successive approximations. Thus have been obtained the curves marked "TISSUE (20)" and "TISSUE (30)" on figure 148.

The researches of Krogh,[118] justify the belief that the outer curves "LUNG (30)" and "TISSUE (30)" more nearly represent the process as it might take place under ideal conditions. But those peculiarities of the blood which are responsible for the wide separation of the diffusion curve for the lung from that for the tissue are in part dependent upon heterogeneous reactions between cells and plasma. Therefore, taking account of the uncertainty regarding the completeness of such reactions during the passage of blood through capillaries, I shall employ the curves labelled (20). In so doing I wish merely to imply that the differences between the diffusion process in the lung and the reverse process in the tissues are probably *at least* as great as these two curves indicate. In any event, it is evident that the cycle marked (20) and that

[118] A. Krogh, Silliman Lectures, New Haven, 1922.

marked (30) are not very unlike. They are, in fact, necessarily similar in all but magnitude; *i.e.,* in radius of curvature. Concerning the effect of inadequate mixing to modify the process, we have no information.

Another similar graphical integration, which will be discussed in the next chapter, makes possible the graduation of the two cycles so as to represent time. The results are rough approximations; even so they can hardly be meaningless. These results are expressed by dividing the time of each process into tenths and marking these divisions on the two cycles.

On each of figures 42 to 146 the cycle (20) has been represented. On the large alignment chart, figure 41, two curves are drawn. These are the envelopes of all lines corresponding to points on the cycle (20). Every tangent to these curves represents some point on cycle (20) of figure 148.

The time scales have also been placed on these envelopes. Thus a tangent at point 0.5 on the lung envelope roughly represents the condition of an infinitesimal portion of a capillary column of blood, when half way through a lung capillary of average dimensions and relations.

Finally, it is interesting to consider the variations in each variable separately, during the cycle. These are represented for the seven principal variables and also, because of anomalous fluctuations, for pH_c, on figures 149 to 156. In each case time is taken as abscissa and the time of passage through the capillary divided into tenths.

With this analysis of the respiratory cycle our formal discussion of the blood is complete. But, before commencing a similar discussion of the relations between the properties of the blood and its respiratory cycle on the one hand, and the variations of the circulation, the respiration, and the metabolism on the other hand, it will be well to consider the accuracy of the quantitative description which has been worked out in the preceding and present chapters.

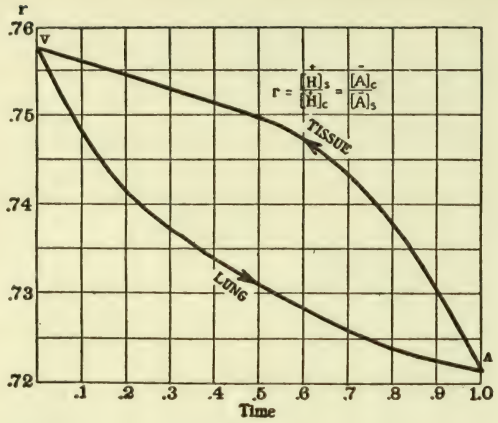

FIG. 149.

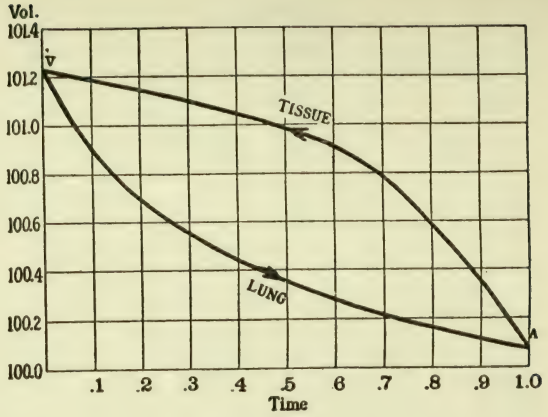

FIG. 150.

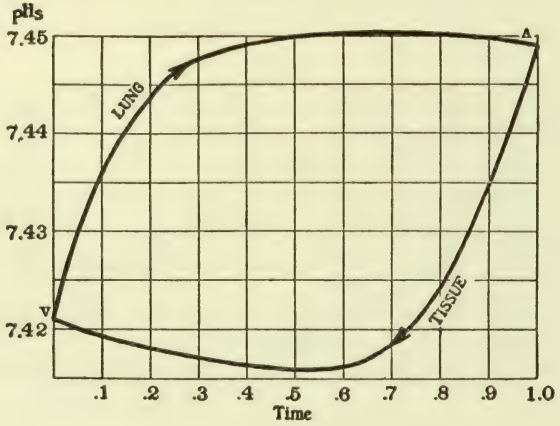

FIG. 151.

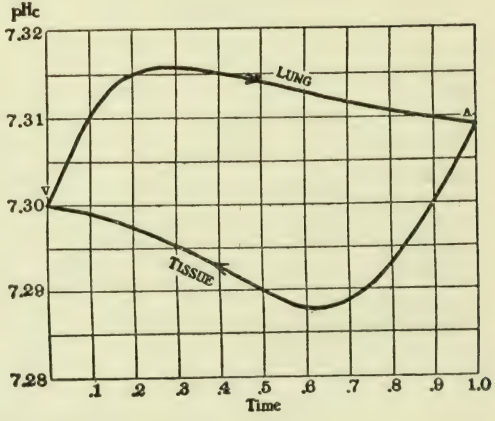

FIG. 152.

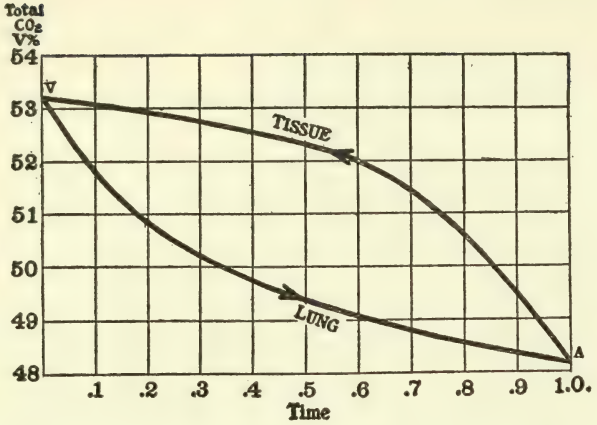

FIG. 153.

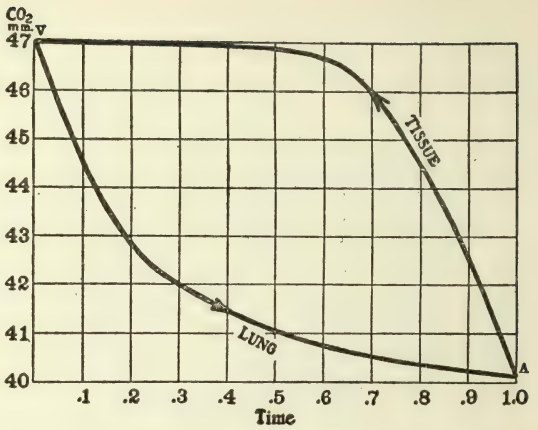

FIG. 154.

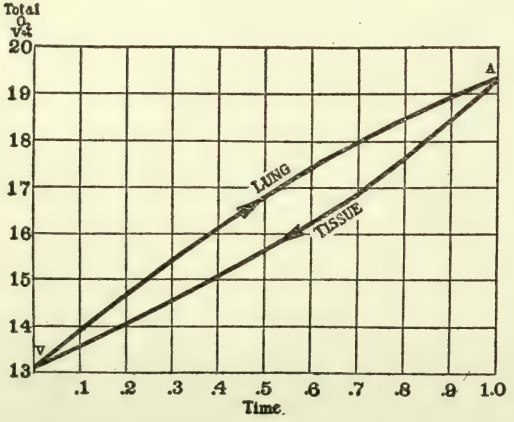

FIG. 155.

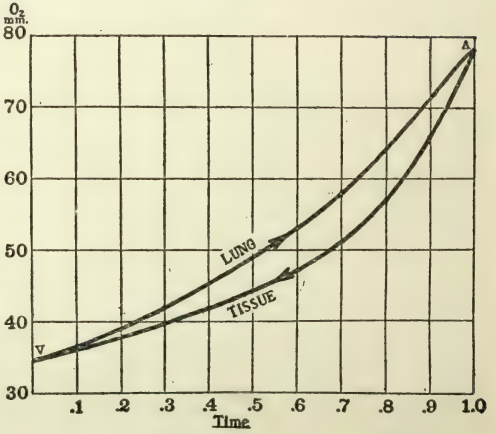

FIG. 156.

In the first place, it should be noted that the experiments, during which all the facts concerning the blood of A.V.B., represented above in graphical and tabular form, were established, avoided an accurate study of the total masses of water, serum protein and base, as well as all measurements of the quantity of the component HX. Accordingly, the estimate of total base neglects the quantity of base required to balance HX and is, therefore, low and in fact equal to B − X. Also, the values for the masses of blood water and of serum protein are appreciably in error. These differences are, for the most part, of no importance, and always of negligible importance for the preceding discussions. They do, however, somewhat affect the inferences which may be drawn from the tables. Further studies of this blood have, therefore, become necessary, although a revision of the figures, with the exception of the complete nomogram, has been unnecessary.

A nomogram for the blood of A.V.B. at rest, revised in accordance with all our present data is accordingly presented as figure 157 and the corresponding estimates of the composition of arterial and venous bloods and of the respiratory cycle are given in tables 19, 20, and 21.[119]

[119] Dill, van Caulaert, Hurxthal, Stoddard, Bock, and Henderson, *Journal of Biological Chemistry,* LXXIII, 251 (1927).

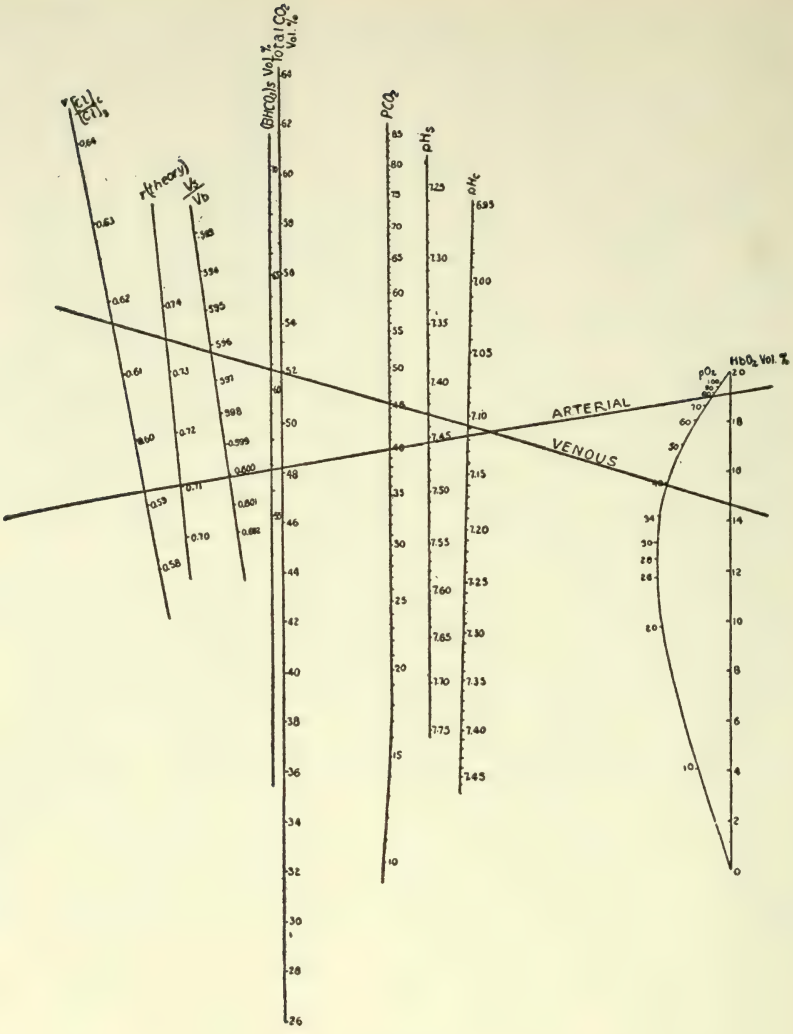

Fig. 157

Blood of A.V.B. at Rest

TABLE 19.

Blood of A.V.B.

Concentration of hemoglobin = 8.93 *mM* per liter of blood.
Concentration of serum proteins = 43.5 gm. per liter of blood.
Respiratory quotient = 0.82.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	550	282	832	546	286	832	−4	+4	0.0
B *mM* " " "	82.71	45.96	128.67	82.71	45.96	128.67			
Cl " " " "	59.59	18.11	77.70	58.72	18.98	77.70	−0.87	+0.87	0.0
BP " " " "	7.88	22.60	30.48	7.80	21.15	28.95	−0.08	−1.45	−1.53
$BHCO_3$ " " " "	15.23	5.25	20.48	16.19	5.83	22.02	+0.96	+0.58	+1.54
$BHCO_3$, *vol. per cent*	34.12	11.77	45.89	36.28	13.06	49.34	+2.16	+1.29	+3.45
H_2CO_3, *mM per l. blood*	0.71	0.34	1.05	0.80	0.39	1.19	+0.10	+0.05	+0.15
H_2CO_3, *vol. per cent*	1.58	0.76	2.34	1.79	0.87	2.66	+0.21	+0.11	+0.32
Total CO_2, *mM per l. blood*	15.94	5.59	21.53	16.99	6.22	23.21	+1.06	+0.63	+1.69
Total CO_2, *vol. per cent*	35.70	12.53	48.23	38.07	13.93	52	+2.37	+1.40	+3.77
Free O_2, *mM per l. blood*			0.09			0.04			−0.04
Free O_2, *vol. per cent*			0.2			0.1			−0.1
Combined O_2, *mM per l. blood*		8.57	8.57		6.56	6.56		−2.01	−2.01
Combined O_2, *vol. per cent*		19.2	19.2		14.7	14.7		−4.5	−4.5
Total O_2, *mM per l. blood*			8.66			6.60			−2.05
Total O_2, *vol. per cent*			19.4			14.08			−4.6
CO_2 pressure, *mm. Hg*			40			45.4			+5.4
O_2 pressure, *mm. Hg*			78			40			−38
Volume, *cc. per l. blood*	600	400		596.2	403.8		−3.8	+3.8	
pH	7.455	7.118		7.429	7.110		−0.026	−0.008	
r_{theory}			0.710			0.735			+0.025
r_{Cl}			0.592			0.616			+0.024

TABLE 20.

A.V.B. Serum.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	916.7	915.8	−0.9
B	*mM* " " "	137.9	138.7	+0.8
Cl	" " " "	99.32	98.49	−0.83
BP	" " " "	13.13	13.08	−0.05
$BHCO_3$	" " " "	25.39	27.16	+1.77
H_2CO_3	" " " "	1.18	1.34	+0.16
Total CO_2	" " " "	26.57	28.50	+1.93

TABLE 21.

A.V.B. Cells.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	705	708.3	+3.3
B	*mM* " " "	114.9	113.8	−1.1
Cl	" " " "	45.27	47	+1.73
BP	" " " "	56.50	52.38	−4.12
$BHCO_3$	" " " "	13.13	14.44	+1.31
H_2CO_3	" " " "	0.85	0.96	+0.11
Total CO_2	" " " "	13.98	15.40	+1.42
Combined O_2	" " " "	21.43	16.25	−5.18

At first sight, the differences between tables 15 and 19 may seem significant. It must be remembered, however, that the definition of the quantity B is not the same in the two cases. For table 15, B is estimated indirectly as the sum of the quantities Cl, BP, and $BHCO_3$, while for table 19 B is determined directly by analysis and it is the quantity X which is indirectly estimated in accordance with the equation,

$$X = B - Cl - BP - BHCO_3.$$

Therefore, we have the value of the B of table 15 equal to that of the B of table 19 minus the value of X, which for table 15 remains undetermined. In this respect table 19 and figure 157 represent merely a closer approximation to a complete treatment of the system, through the introduction of component 4a of Chapter II. This procedure,

otherwise of little moment, has one advantage. It throws into the value of the quantity X of the table most of the effects of errors of experiment and of theoretical approximation and thus affords a rough indication of the trustworthiness of the conclusions. For example, it will be seen from table 19 that X appears to move between cells and plasma during the respiratory cycle in the direction opposite to that followed by chloride. But it is extremely improbable that this should be the fact. Hence we may attribute to the variations of the value of X errors of at least a few tenths of a millimole.

As above suggested, the *total* quantities of X, of B, of H_2O, and of P_s are of small importance, and it is for this reason that no attempt was made to estimate them correctly in constructing table 15.

If we turn our attention to the variables which are important for our present purposes, it will be found that differences between the two tables depend upon one difference only in the conditions. This is the difference in amplitude of the respiratory cycle, which for table 15 corresponds to a sensibly higher metabolic rate and coefficient of utilization of oxygen than for table 19. The latter table describes the condition of rest. The disparity between the two tables is more easily to be seen by an analysis of their last three columns. In the first three columns of the second table, the difference also depends on the presence of X and the variations of H_2O and of B already discussed. To this statement there are but two exceptions of any importance: (1) in accordance with the observation of Van Slyke, Hastings, Murray and Sendroy[120] the value $pk' = 5.93$ has been employed in calculating the value of pH_c and (2) measurements of somewhat uncertain accuracy for the values of the r's have led to other slight modifications of the values which appear in the earlier tables. Since the values of the last three columns of the tables depend upon the positions of the arterial and ve-

[120] Van Slyke, Hastings, Murray, and Sendroy, *Journal of Biological Chemistry,* LXV, 701 (1925).

nous blood lines, while the values of total water, total base, serum protein, and even the presence of X are without sensible effect, the nomogram remains essentially unchanged.

As before, the total change in $BHCO_3$ for whole blood is equal to that in BP, but with the component HX added to the description, it is necessary, in seeking an interpretation of changes in serum and cell bicarbonates, to take account of variations in concentration of both X and Cl for the two phases. The share of serum protein in the transport of carbonic acid remains a mere five per cent, the hemoglobin being still accountable for 95 per cent of all the carbonic acid carried and, as before, nearly seven-eights of this is attributable to the oxygen effect. The magnitude of the inverse action, which may be called the carbonic acid effect upon oxygen transport, is more difficult to define and will be discussed at length in Chapter IX. It is much smaller in magnitude; measured in millimoles, about one-fourth the oxygen effect; measured relatively to the total amount of material transported, about one-fifth.

The change in total concentration of ions plus undissociated molecules between arterial and venous bloods, which may be roughly estimated by the difference between the change in total carbonic acid and the change in free oxygen, amounts to 1.64 millimoles per liter blood, or 1.93 millimoles per liter water. Of this 1.80 millimoles per liter water is attributable to bicarbonate. In the serum, the sum of the changes in base, X, chloride, and bicarbonate concentrations per liter water is 1.93 millimoles; in the cells 1.94. Obviously, these values should be nearly equal to each other and to the change in bicarbonate per liter blood water. They are, however, greatly modified by very small variations in the estimates of the distribution of water between cells and serum. For example, if the movement of water between the two phases in the respiratory cycle be estimated as 4 cc. per liter blood instead of 3 cc. as in the table and nomogram, the

change in electrolyte concentration per liter cell water appears to be only about 1.0 millimole, that per liter serum water, however, 2.4 millimoles. Similar effects may be demonstrated for small variations in the estimates of the *r*'s. Since it is hardly possible to measure changes of volume or of *r* with the accuracy necessary to obtain consistency between all the values of the tables, it is necessary to use a method of successive approximations in order to find the most consistent values of the variables. To such refinements of computation little importance should be attributed, for theory must not be pressed too far in estimating minute differences and, unless theory is to be relied upon, such a procedure amounts to nothing more than "rigging" the data. For this reason, in the pages which follow meaningless precision and consistency in the nomograms and tables have not been sought.

The total ionic concentration of arterial blood serum ($B + X + Cl + BHCO_3$) is 312.5 millimoles per liter water, that of arterial blood cells 299.4 millimoles per liter water. The difference may be due to differences between the two phases in activity coefficients of water and of dissolved substances, as well as to the relatively large osmotic effect of hemoglobin, and to unequal distributions of substances not accounted for in the balance sheet.

There can be but little profit in further analysis of the nomogram and of the tables. It will be found in the succeeding chapters that they meet the test of use and that they appear to be free from large errors, except, perhaps, such as may be involved in the inaccuracy of accepted values of physical and chemical constants used in computation. But when errors of this kind are discovered they can ordinarily be corrected.

More useful is comparison with the nomogram and tables representing the blood and respiratory cycle of another normal man, C.V.C., at rest. These may be found in figure 158 and in tables 22, 23, and 24.[121] In this case

[121] Dill, van Caulaert, Hurxthal, Stoddard, Bock, and Henderson, *Journal of Biological Chemistry,* LXXIII, 251 (1927).

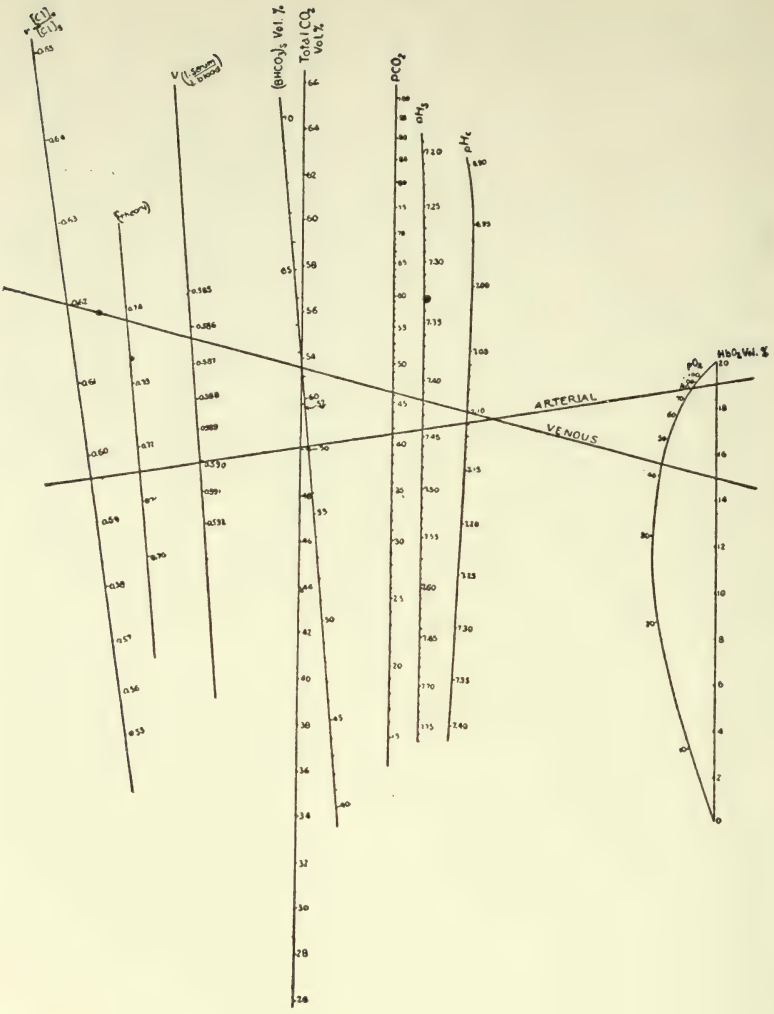

Fig. 158

Blood of C.V.C. at Rest

TABLE 22.

Blood of C.V.C.

Concentration of hemoglobin = 8.93 *mM* per liter of blood.
Concentration of serum proteins = 40.0 gm. per liter of blood.
Respiratory quotient = 0.82.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	544	293	837	540	297	837	−4	+4	0.0
B *mM* " " "	83.02	47.49	130.51	83.02	47.49	130.51			
Cl " " " "	60.51	19.28	79.79	59.82	19.97	79.79	−0.69	+0.69	0.0
BP " " " "	7.22	22.18	29.4	7.13	20.87	28	−0.09	−1.31	−1.4
$BHCO_3$ " " " "	15.29	6.03	21.32	16.07	6.65	22.72	+0.78	+0.62	+1.4
$BHCO_3$, *vol per cent*	34.25	13.52	47.77	36	14.93	50.93	+1.75	+1.41	+3.16
H_2CO_3, *mM per l. blood*	0.73	0.36	1.09	0.81	0.41	1.22	+0.08	+0.05	+0.13
H_2CO_3, *vol. per cent*	1.63	0.8	2.43	1.81	0.91	2.72	+0.18	+0.11	+0.29
Total CO_2, *mM per l. blood* ..	16.02	6.39	22.41	16.88	7.06	23.94	+0.86	+0.68	+1.54
Total CO_2, *vol. per cent*	35.88	14.32	50.2	37.81	15.84	53.65	+1.93	+1.52	+3.45
Free O_2, *mM per l. blood*			0.09			0.04			−0.04
Free O_2, *vol. per cent*			0.2			0.1			−0.1
Combined O_2, *mM per l. blood*		8.53	8.53		6.7	6.7		−1.83	−1.83
Combined O_2, *vol. per cent* ..		19.1	19.1		15	15		−4.1	−4.1
Total O_2, *mM per l. blood*			8.62			6.74			−1.88
Total O_2, *vol. per cent*			19.3			15.1			−4.2
CO_2 pressure, *mm. Hg*			41.5			46.4			+4.9
O_2 pressure, *mm. Hg*			79			43			−36
Volume, *cc. per l. blood*	590	410		586.3	413.7		−3.7	+3.7	
pH	7.442	7.108		7.416	7.098		−0.026	−0.01	
r_{theory}			0.715			0.739			+0.024
r_{Cl}			0.596			0.620			+0.024

TABLE 23.

C.V.C. Serum.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	922	921	−1
B	*mM* " " "	140.7	141.6	+0.9
Cl	" " " "	102.56	102.03	−0.53
BP	" " " "	12.24	12.16	−0.08
$BHCO_3$	" " " "	25.92	27.41	+1.49
H_2CO_3	" " " "	1.24	1.38	+0.14
Total CO_2	" " " "	27.16	28.79	+1.63

TABLE 24.

C.V.C. Cells.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	714.7	717.9	+3.2
B	*mM* " " "	115.83	114.79	−1.04
Cl	" " " "	47.03	48.27	+1.24
BP	" " " "	54.1	50.45	−3.65
$BHCO_3$	" " " "	14.71	16.08	+1.37
H_2CO_3	" " " "	0.88	0.99	+0.11
Total CO_2	" " " "	15.59	17.07	+1.48
Combined O_2	" " " "	20.81	16.19	−4.38

total base was not determined. X is, therefore, unaccounted for and the value of B, equal to BP + $BHCO_3$, is correspondingly low. Between the blood of C.V.C. and that of A.V.B. there is, during rest, no significant difference. The respiratory cycles are also indistinguishable. Therefore, since observations on several other normal men agree closely with these two cases, it seems probable that the facts presented in this and the preceding chapters may be used with confidence as a basis for comparative studies, including a comparison of rest and activity, of health and disease, and of species and species. Our studies have not yet gone far enough to enable us to define the range of variation which may be regarded as normal in man at rest. But it seems probable that normal variation involves little more than well-known minor fluctuations in the volumes of the two phases of the blood and in the level

of the carbon dioxide dissociation curves. These result in merely trivial modifications of the nomogram and of the respiratory cycle.

All the figures and tables of the preceding chapter and of the present one may be used as a means of defining the system of the blood and its activities. The figures of the preceding chapter, together with tables 15, 16, and 17 above, are more comprehensive and more convenient. The respiratory cycle which they define is one of moderate activity. On the other hand, the second characterization of the blood of A.V.B., which the present chapter presents, corresponds to the state of rest. In some respects it is slightly more accurate than the first description; in other respects it makes use of slightly different constants, which at present seem preferable to those formerly employed; and, for these reasons, it appears to be better fitted for use as a standard of comparison.

CHAPTER VIII

BLOOD AND CIRCULATION

A STATE of mutual dependence exists, not only between the different functions of the blood, but also between these functions and those of the circulation and respiration, and the other physiological activities of the individual. It is, therefore, possible to deduce from the nomographical description of the blood certain conclusions regarding the circulation and other physiological functions. But, needless to say, the properties of the blood cannot unequivocally determine those of the circulation; they do but impose certain restrictions upon these properties. It is these restrictions that we shall now study.

In the preceding chapter we have obtained, by the use of a graphical method, an estimate of the sequence of changes in the blood which constitute its respiratory cycle. The method makes use of experimental data and of the single assumption that the movements of oxygen and of carbonic acid into the blood and out of it are processes of diffusion, not complicated by unknown phenomena. The accuracy of the method is somewhat uncertain, because the coefficients of diffusion of oxygen and carbonic acid within the body can be only roughly estimated, and because we are not fully informed concerning the rate of mixing of blood in capillaries. But there is little reason to doubt the substantial trustworthiness of the result, which is represented by the cycle of figure 148 and by that of the large nomogram, figure 41. For the determination of time as a function of change in the composition of blood a second graphical method has been employed in these calculations, the result of which is given in a simple form on figure 148, and also on figure 41. To this method, which has not yet been described, we must first turn our attention.

In order to fix our ideas we may begin with a tabulation of simultaneous values of the concentrations of free oxygen and of oxyhemoglobin, throughout the cycle represented by figure 148.[122]

TABLE 25.

HbO_2 saturation	pO_2	
	Lung	Tissues
per cent	*mm.*	*mm.*
65	34.0	34.0
70	37.0	37.4
75	40.5	41.3
80	44.7	45.7
85	50.0	51.4
90	58.0	61.2
95	73.2	75.0
96	78.0	78.0

Taking first the case of the lung, it is evident that table 25 makes possible the calculation, for every value of HbO_2, of the effective head of oxygen pressure causing the diffusion of oxygen from alveolar air to blood. This head of pressure is, in fact, equal to the mean partial pressure of oxygen in the alveolar air, minus the partial pressure of free oxygen in blood. Thus it is possible by simple subtraction, given the partial pressure of oxygen in the alveolar air of A.V.B. as 110 mm., to obtain the values of table 26.

TABLE 26.

Lung.

HbO_2 saturation	O_2 head (Δp)
per cent	*mm.*
65	76.0
70	73.0
75	69.5
80	65.3
85	60.0
90	52.0
95	36.8
96	32.0

[122] Henderson and Murray, *Journal of Biological Chemistry,* LXV, 407 (1925).

Making use of this table, we may next lay off as ordinates on figure 159 values of HbO_2, and at convenient intervals draw sets of parallel lines whose slopes measure the head of oxygen pressure for the ordinates on which they are placed. Then that curve which, beginning at $HbO_2 = 66$ per cent and ending at $HbO_2 = 96$ per cent, is everywhere parallel, at the corresponding values of HbO_2, with the slopes thus drawn, represents the necessary course of the diffusion process in the lung. Therefore, if we assume uniformity of structure and of blood flow in the capillary, the abscissa under the curve may be taken to represent the length of an average lung capillary, or, what comes to the same thing, time.

This may be stated mathematically as follows. Let R be rate of diffusion; Δp, head of oxygen pressure; s, quantity of oxygen diffusing; and t, time. Then

$$R = k\Delta p = \frac{ds}{dt}.$$

Accordingly, it is only necessary to divide the abscissa under the curve into ten equal parts and to read from the curve the values of HbO_2 corresponding to these divisions, in order to be able to graduate the process of diffusion in the lung in the manner of the preceding chapter.

On the assumption that the oxygen concentration in the tissues is negligibly small, and taking into consideration an average capillary, which delivers blood of the composition of mixed venous blood to the vein, it is easy to repeat the construction and thus to obtain a corresponding result for a capillary of the greater circulation. This has been done in figure 160. Here also one is dealing with an ideal capillary corresponding to a statistical mean, and in this case, no doubt, the extreme departures from the mean which actually occur in the organism are very large. It is also extremely improbable that in all parts of the body the oxygen concentration of the tissues can be negligibly small. We shall return to a consideration of these difficulties.

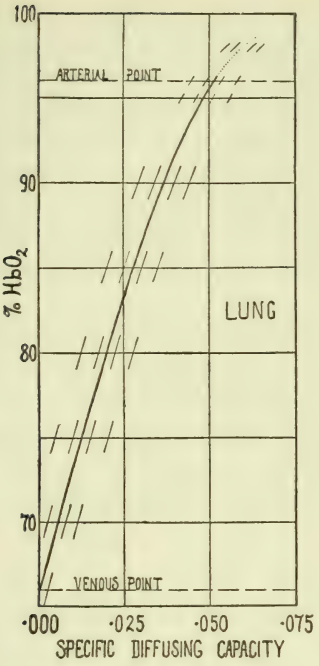

Fig. 159
Diffusion in Lung

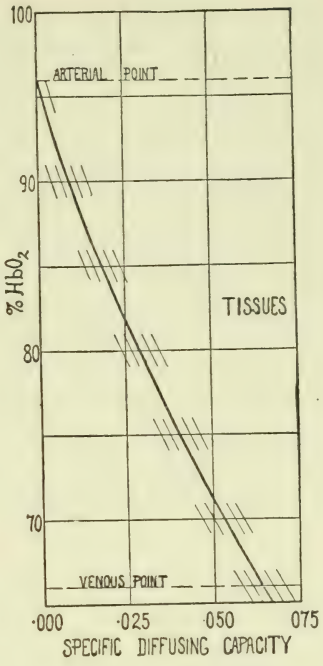

Fig. 160
Diffusion in Tissues

From what has been said above it is evident that we may draw useful conclusions from a comparison of the lengths of the abscissas under the curves of figures 159 and 160. These lengths are, in fact, proportional to the total areas of uniform diffusing surface over which, in equal periods of time, equal volumes of arterial and venous blood must pass, under the conditions which have been assumed in the construction of the figures, in order that the respiratory exchange may be accomplished. Each length may be regarded as a measure of the *specific diffusing capacity* of the capillary system in question, in other words of the diffusing capacity, per liter of blood flow, per minute, of the capillaries of the lesser and of the

greater circulations respectively. Their ratio measures, therefore, the relative diffusing capacities of lung and tissue capillaries for the conditions now under discussion. We may draw the conclusion that in a normal individual, under conditions which exist during moderate exercise, when about one-third of the oxygen content is removed from the blood in its passage through the greater circulation, the diffusing capacity of the active capillaries of the greater circulation must be at least 20 per cent greater than that of the active capillaries of the lung.

There is no difficulty in repeating this investigation for other respiratory cycles. At present it will perhaps suffice to restrict our attention to three other instances in which the arterial point as well as the respiratory quotient remain unchanged, while the venous points fall at 81, 50, and 11 per cent of HbO_2 respectively. Figure 161 gives

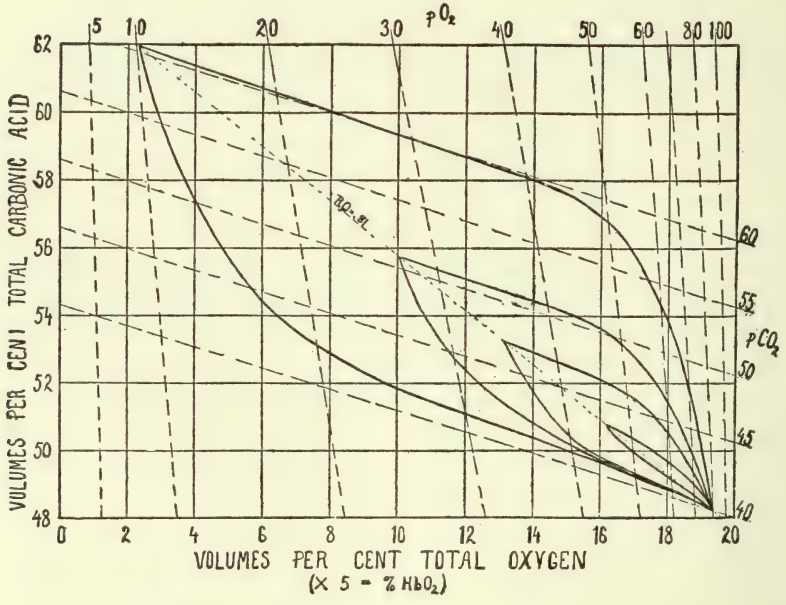

Fig. 161

Comparison of Respiratory Cycles

the result of the first integration for these three cases, and also for the original case. Once more the oxygen of the alveolar air is assumed to be at a pressure of 110 mm., that in the tissues at 0 mm.

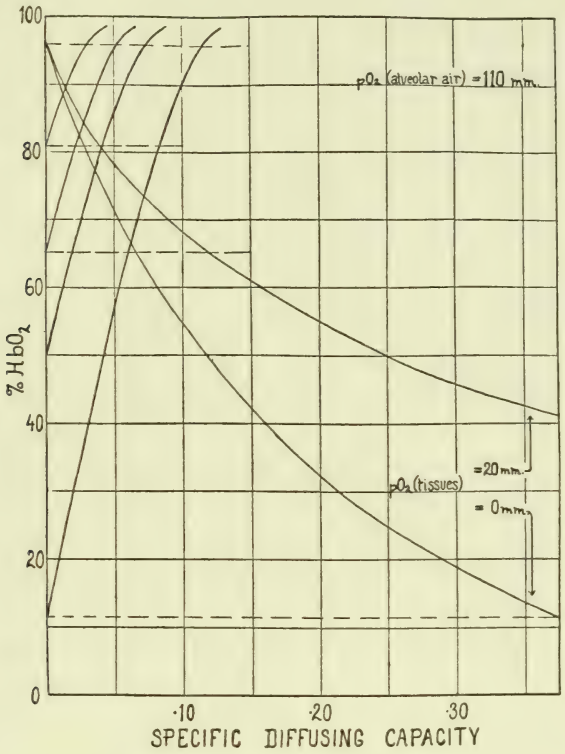

Fig. 162

Comparison of Conditions of Diffusion

In figure 162 the results of the second integration for all four cycles are represented. In addition there is given the result obtained on the assumption that the pressure of oxygen in the tissues of the greater circulation is 20 mm. From this figure it is possible to read the values of

the specific diffusing capacity for eleven cases. These are assembled in table 27.

TABLE 27.

Specific diffusing capacity (arterial point = 96 per cent of HbO_2).

Venous point, per cent of HbO_2	81	66	50	11
Lung ($pO_2 = 110$)	0.030	0.051	0.072	0.114
Tissues ($pO_2 = 0$)	0.027	0.063	0.118	0.375
Tissues ($pO_2 = 20$)	0.038	0.120	0.250	

TABLE 28.

Diffusing capacity per liter of oxygen per minute (arterial point = 96 per cent of HbO_2).

Venous point, per cent of HbO_2	81	66	50	11
ΔO_2 per cent	15	30	46	85
Lungs ($pO_2 = 110$)	1.00	0.85	0.79	0.67
Tissues ($pO_2 = 0$)	0.90	1.05	1.29	2.21
Tissues ($pO_2 = 20$)	1.27	2.00	2.74	

More clearly to illustrate the conditions imposed upon the organism by the properties of the blood, the data of table 27 have been converted into those of table 28. Here are presented the values of diffusing capacity, per liter of oxygen diffusing, per minute.

These tables lead to the conclusion that the total area of diffusing surface, which we may assume to be roughly proportional, for similar structures, to the number of physiologically active capillaries, is subject to wide variation. Such variation is the expression of a simple physical necessity.

The coefficient of utilization of oxygen seems never to rise in normal man far above 60 per cent, while, in a stationary state of heavy work, oxygen consumption may be ten or even fifteen-fold greater than during rest. It is evident, accordingly, that diffusing capacity in the lungs may be ordinarily subject to a tenfold increase and in the tissue to a far greater increase. Precise estimates are

impossible without knowledge of the variations of oxygen partial pressure in lungs and tissues, while the problem of the distribution of the blood to the different parts of the body raises difficulties of another character, in part statistical, and there are also further complications.

The root of the matter is no other than that which confronted us when studying separately the acid-base equilibrium and dissociation curves and the heterogeneous equilibrium of the blood. For at another level of physiological integration we are once more seeking the relations between a number of variables such as diffusing capacity, coefficient of utilization of oxygen, head of oxygen pressure, blood flow, and metabolic rate. Once more each of these variables is a function of all the others. Given the same difficulty, a similar resolution should be sought and it has been found by Murray and Morgan.[123] Therefore, we must next follow their treatment of the subject.

We shall begin with a series of definitions. Let the metabolic rate, MR, be measured by the consumption of oxygen in cubic centimeters per minute; the blood flow, BF, in liters per minute, the concentration of hemoglobin in the blood, Hgb, in volumes per cent, the coefficient of utilization of oxygen, ΔO_2 per cent, by the difference in per cent saturation of hemoglobin in arterial and of venous bloods. Let ΔO_2 cc. be the difference, measured in cubic centimeters, between the oxygen content of one cubic centimeter of arterial and venous bloods. Let DC be the diffusing capacity, SDC the specific diffusing capacity, and $\Delta p'$ the head of pressure, measured in cubic centimeters of mercury, which, if the head were constant throughout the capillary, would involve the diffusion of oxygen that does in fact take place under the varying head of pressure.

Then we have, from the law of diffusion

$$\Delta p' \times \mathrm{DC} = \mathrm{MR}, \qquad (1)$$

[123] Murray and Morgan, *Journal of Biological Chemistry,* LXV, 419 (1925).

where the unit of DC, twenty times greater than that above employed, is determined by the relation expressed in equation 1.

Also, from the definition of specific diffusing capacity, we have

$$10\Delta p' \times \text{SDC} = \Delta O_2 \text{ per cent},$$

or if we follow Murray and Morgan and define a new factor, SHF, the specific hemoglobin flow by the relation

$$\text{SHF} = \frac{1}{\text{SDC}},$$

we have

$$10\Delta p' = \text{SHF} \times \Delta O_2 \text{ per cent}, \tag{2}$$

and, finally, the two following relations are self-evident:

$$\Delta O_2 \text{ per cent} \times \text{Hgb} = 10{,}000\ \Delta O_2 \text{ cc.}, \tag{3}$$

$$1000\ \Delta O_2 \text{ cc.} \times \text{BF} = \text{MR}. \tag{4}$$

The meaning of the term $\Delta p'$ may be more readily appreciated by reference to figure 159. On this figure a straight line drawn from the venous point to the arterial point has a slope $\Delta p'$, which is related to the slope of the small lines measuring the values of Δp for different points in time in accordance with the equation

$$\Delta p' \times t = \int_{t_v}^{t_A} \Delta p dt.$$

This factor, $\Delta p'$, has been previously considered by Bohr[124] and by Barcroft.[125]

The meaning of the term DC can hardly be defined with precision, except by means of the discussion of the earlier portion of this chapter. Certain it is, however, that, all

[124] C. Bohr, *Skandinavisches Archiv für Physiologie,* XXII, 221 (1909).

[125] Barcroft, Binger, Bock, Doggart, Forbes, Harrop, Meakins, and Redfield, *Philosophical Transactions, Royal Society,* CCXI, 351, London, 1922.

other things being equal, it must be proportional to the number of open capillaries and this is perhaps for similar organs often approximately true, since we may expect statistical uniformities to manifest themselves.

Each of the equations 1, 2, 3, and 4, may be represented by a simple Cartesian contour line chart on which the contour lines radiate from the origin. Moreover, the fact that some variables occur twice in the equations makes possible an arrangement of four adjoining graphs in such a way that but two sets of ordinates (MR and ΔO_2 per cent) and two sets of abscissas ($\Delta p'$ and ΔO_2 cc.) are employed. This arrangement will be found on figure 163.

On this figure the lower right quadrant corresponds to equation 2, where the Cartesian coördinates represent $\Delta p'$ and ΔO_2 per cent, the radiating contour lines SHF, the value of which, it will be remembered, is equal to $\frac{1}{SDC}$. The lower left quadrant corresponds to equation 3, and the radiating lines represent values of Hgb. The upper right quadrant corresponds to equation 1, with radiating lines for the values of DC. Finally, the upper left quadrant corresponds to equation 4 and its radiating lines give values of BF.

On figure 163 the relations between the variables now in question may be conveniently and at the same time completely represented. As an example, let us take the case where the value of MR is 210 cc. O_2 per minute, of Hgb 20 volumes per cent, of ΔO_2 per cent 21 per cent. By the process of integration described above $\Delta p'$ for the lung is found to have the value 53 mm. We now draw horizontal lines through the ordinates corresponding to MR = 210 and ΔO_2 per cent = 21, next a vertical line through the abscissa corresponding to $\Delta p' = 53$ and finally a vertical line through the point of intersection of the ordinate corresponding to ΔO_2 per cent = 21 with the contour line corresponding to Hgb = 20. In this manner a rectangle is constructed. The positions of the four

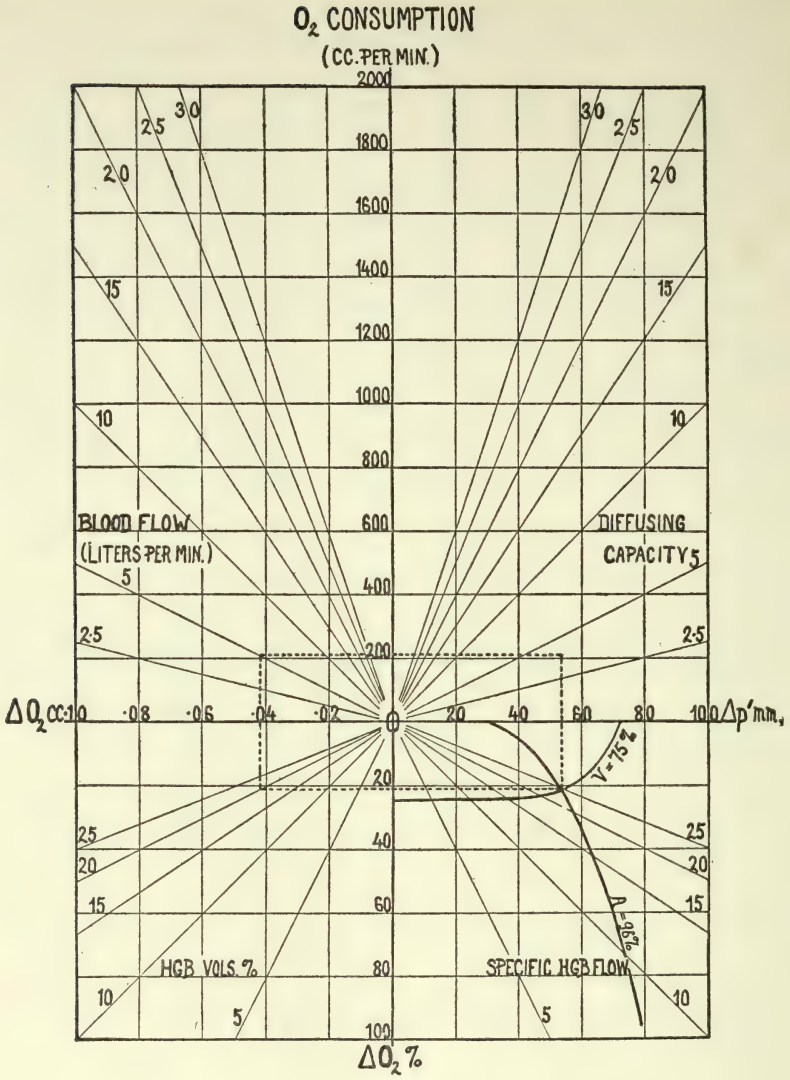

Fig. 163

Nomogram of Murray and Morgan

angles of this rectangle give the values of all the other variables for the lung as follows:

$$\begin{aligned} \text{SHF} &= 25\ (\text{SDC} = 0.040), \\ \Delta O_2\ \text{cc.} &= 0.042, \\ \text{BF} &= 5, \\ \text{DC} &= 4.0. \end{aligned}$$

Such a method of presenting the facts is, however, of but little value and the real usefulness of the figure depends upon the construction of a set of auxiliary charts which obviate the laborious estimates of the values of $\Delta p'$ or of the diffusing capacity, and make possible the determination of all the eight variables from measurements, in the case of the pulmonary circulation, of the values of MR and Hgb and in addition of pO_2 for alveolar air, of pH_s for arterial blood and of the oxygen content of arterial and venous bloods. A similar procedure is also available as a means of defining the conditions of the greater circulation.

We have seen that it is possible to determine the values in the lung of DC, $\Delta p'$, and also of SDC, from complete information concerning a given specimen of blood, when the pressure of oxygen in alveolar air and the arterial and venous points are known. Accordingly, this may be done for a large number of cases, thus enabling us by interpolation to find any of these values without computation, in any case which may later present itself. The problem which Murray and Morgan have solved is how to make such interpolation convenient. Their first step is to reduce the problem to that of finding the position of that angle of the rectangle which falls in the lower right quadrant of figure 163. Clearly, this is a convenient simplification, for the position of this point, the value of MR, and the value of Hgb determine the rectangle. Their next step depends upon the fact that the most important variables in determining the differences between different physiological states are the hydrogen ion concentration of the

cells and the oxygen pressure of alveolar air, or, in the case of the greater circulation, the corresponding oxygen pressure of the tissues. However, the values of pH_c are not very easy to measure, while they are in general, for normal human blood, not far from proportional to values of pH_s. Therefore, values of pH_s, of alveolar pO_2, and of tissue pO_2 have been chosen as the basis of the interpolation.

Finally a series of charts, twenty-one in number, have been constructed, corresponding to conveniently spaced values of pH_s and of pO_2 for alveolar air or for the tissues. Each of these charts is to be regarded as a lower right quadrant of figure 163. Each contains for a particular value of pH_s and of pO_2 in alveolar air, or tissues, contour lines which designate the oxygen contents of arterial and venous bloods. The position of these lines has been calculated in accordance with the methods explained at the beginning of this chapter. In every case the intersection of the venous line corresponding to the oxygen content of venous blood with the arterial line corresponding to the oxygen content of arterial blood determines the position of the lower right angle of the rectangle on figure 163. Thus each chart is based on the simplifying assumption that for given values of pH_s and of pO_2 in alveolar air or tissues the positions of the arterial and venous points unequivocally determine the conditions of diffusion, regardless of fluctuations in Hgb and in other components of the blood. As an approximation this assumption is justified by all our experience with normal human blood. In pernicious anemia, in the two cases of nephritis which we have studied, and in the turtle, a sensible modification of the relation between pH_s, pH_c, and the affinity of hemoglobin for oxygen seems to exist. This slightly modifies the method of using the charts.

Aside from the use of figure 163 and of the twenty-one alternative lower quadrants of that figure as a means of defining the conditions in a particular case, these graphical constructions afford an invaluable aid in understand-

ing the relations between the following variables: (1) oxygen saturation of arterial blood, (2) oxygen saturation of venous blood, (3) partial pressure of oxygen in alveolar air, (4) partial pressure of oxygen in tissues, (5) pH_s, (6) metabolic rate, (7) blood flow, (8) diffusing capacity, (9) mean head of oxygen pressure in the capillaries, (10) oxygen transport per unit volume of blood, (11) variation in saturation of hemoglobin with oxygen, (12) hemoglobin content of blood, (13) specific hemoglobin flow or specific diffusing capacity. Many of these relations are, indeed, simple and obvious, but when so many are simultaneously involved an artificial aid to the imagination is indispensable.

The twenty-one alternative lower quadrants of figure 163, charts I to XXI, are arranged according to the following plan:

pH_s	Lung charts. pO_2 alveolar air.					Tissue charts. pO_2 tissues.	
	110	100	90	70	50	0	20
7.25	I	II	III	IV	V	VI	VII
7.45	VIII	IX	X	XI	XII	XIII	XIV
7.65	XV	XVI	XVII	XVIII	XIX	XX	XXI

The scale for $\Delta p'$, in mm. of mercury, is numbered at the top of each chart. ΔO_2 per cent, the scale of ordinates, is measured downward. On each of the lung charts is a set of curves, numbered at their lower extremities according to the arterial percentage saturations, A per cent, which they represent. These lines are crossed by a few venous lines, and limited by the curve V per cent = 0. The limit of arterial saturation is given by the numbers in brackets (near the numeral designating the number of each chart), and this limit is represented by the vertical line $\Delta p' = 0$. On the tissue charts the general positions of the arterial and venous lines are reversed, and the limit of reduction of the blood is given as before in brackets, and this limit also is represented by the line $\Delta p' = 0$.

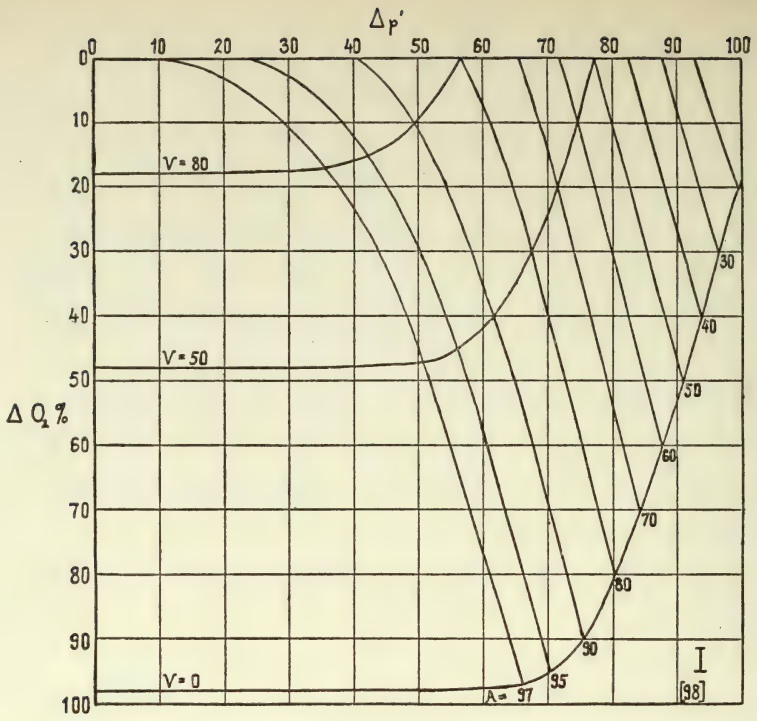

CHART I. pO_2 alveolar air = 110 mm.; pH, = 7.25.

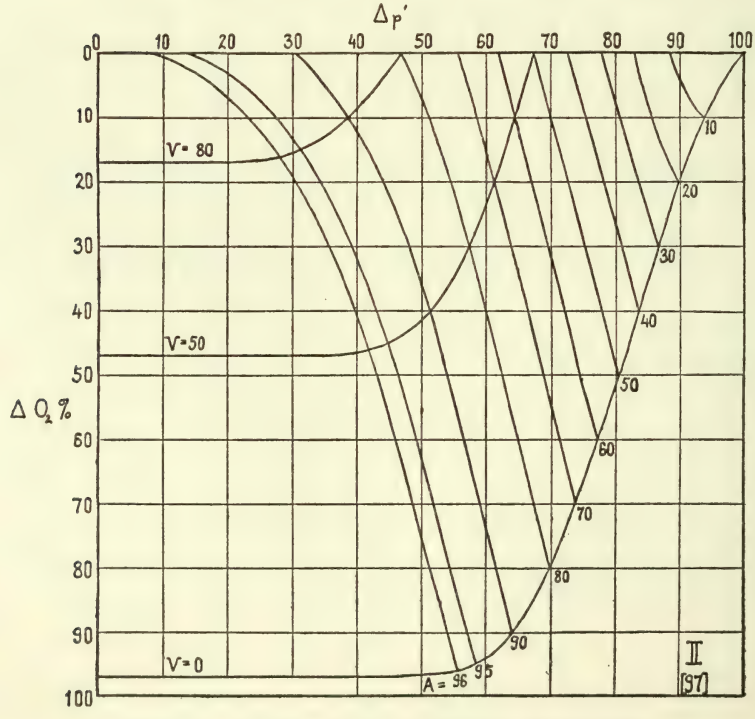

CHART II. pO_2 alveolar air = 100 mm ; pH_a = 7.25.

Fig. 164 *and Fig.* 165

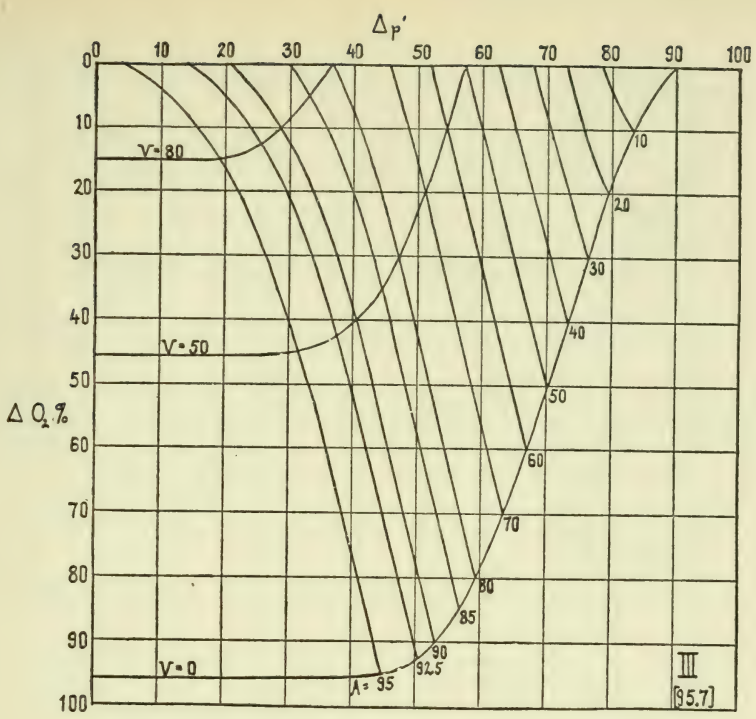

CHART III. pO_2 alveolar air = 90 mm.; $pH_s = 7.25$.

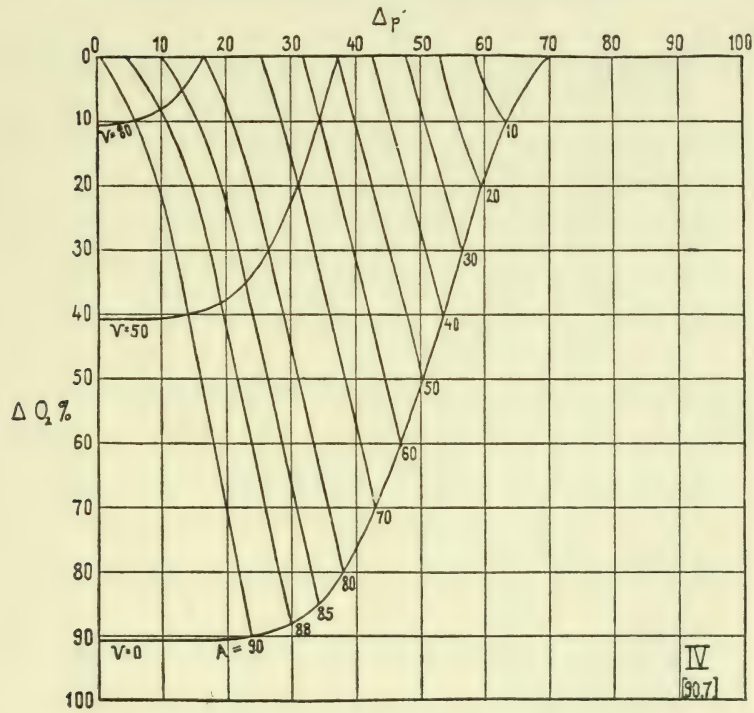

CHART IV. pO_2 alveolar air = 70 mm.; $pH_s = 7.25$.

Fig. 166 *and Fig.* 167

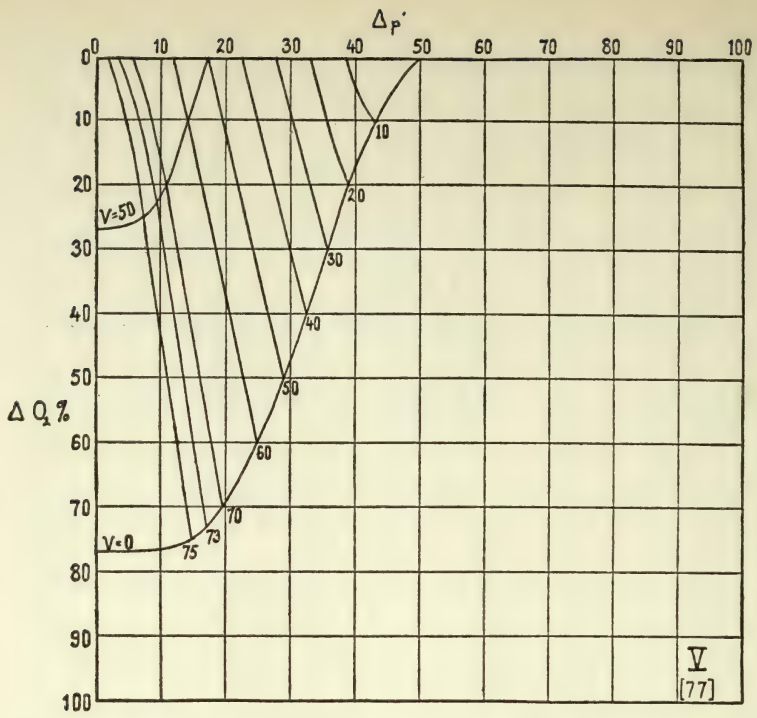

CHART V. pO_2 alveolar air = 50 mm.; pH_s = 7.25.

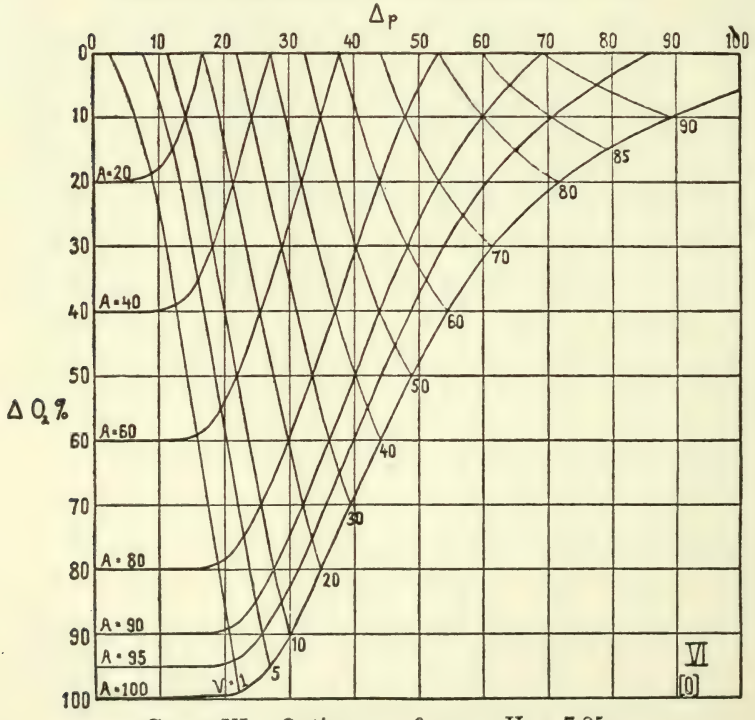

CHART VI. pO_2 tissues = 0 mm.; pH_s = 7.25.

Fig. 168 *and Fig.* 169

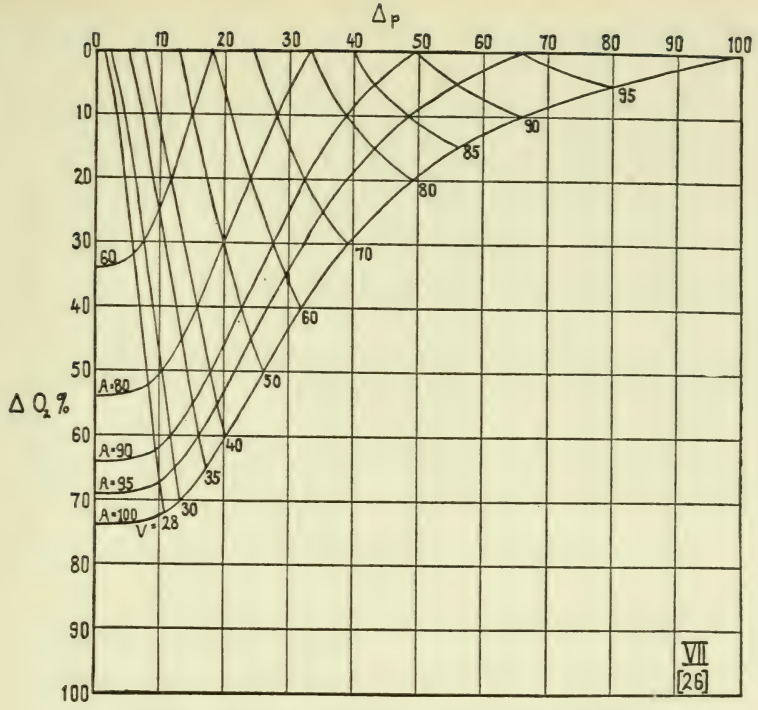

CHART VII. pO_2 tissues = 20 mm.; pH_s = 7.25.

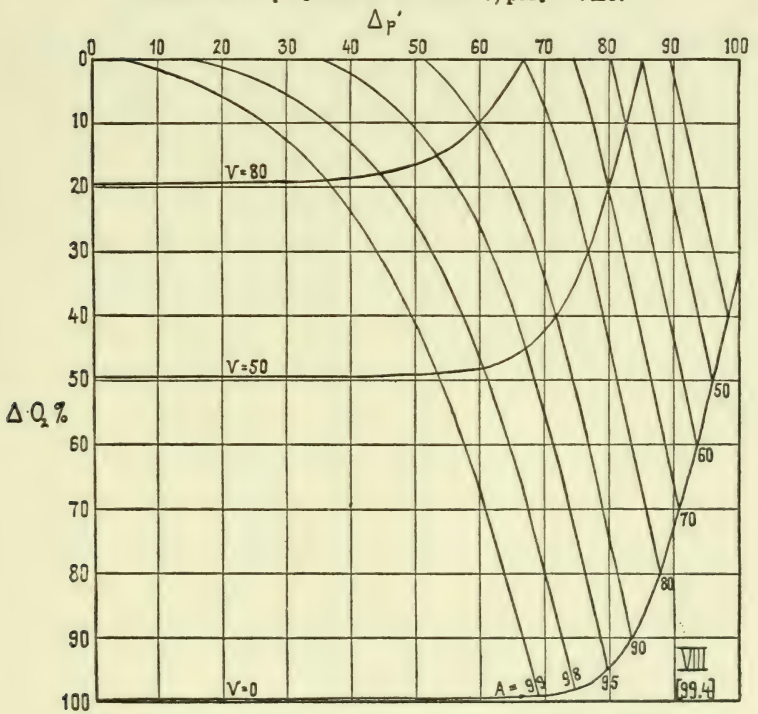

CHART VIII. pO_2 alveolar air = 110 mm.; pH_s = 7.45.

Fig. 170 *and Fig.* 171

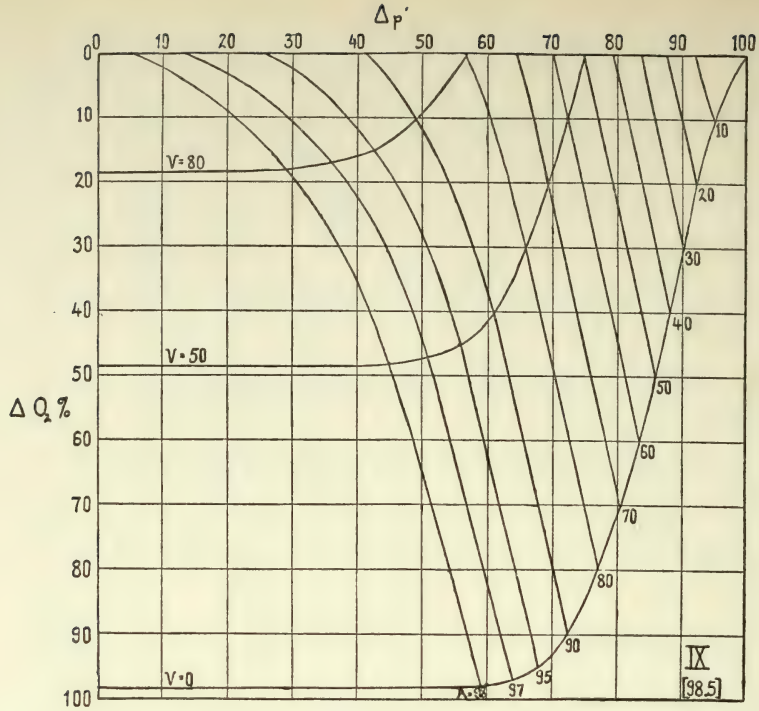

CHART IX. pO_2 alveolar air = 100 mm., pH_s = 7.45.

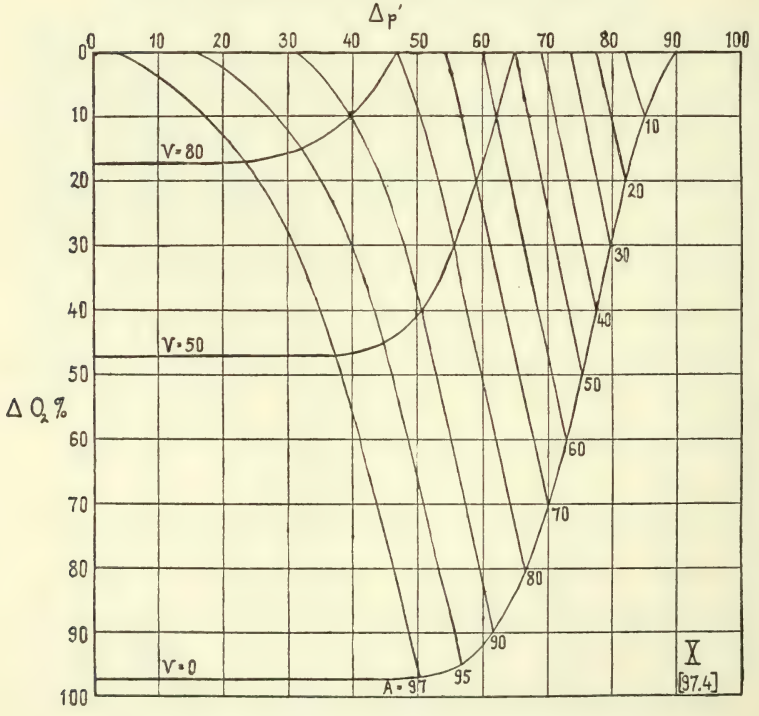

CHART X pO_2 alveolar air = 90 mm.; pH_s = 7.45

Fig. 172 *and Fig.* 173

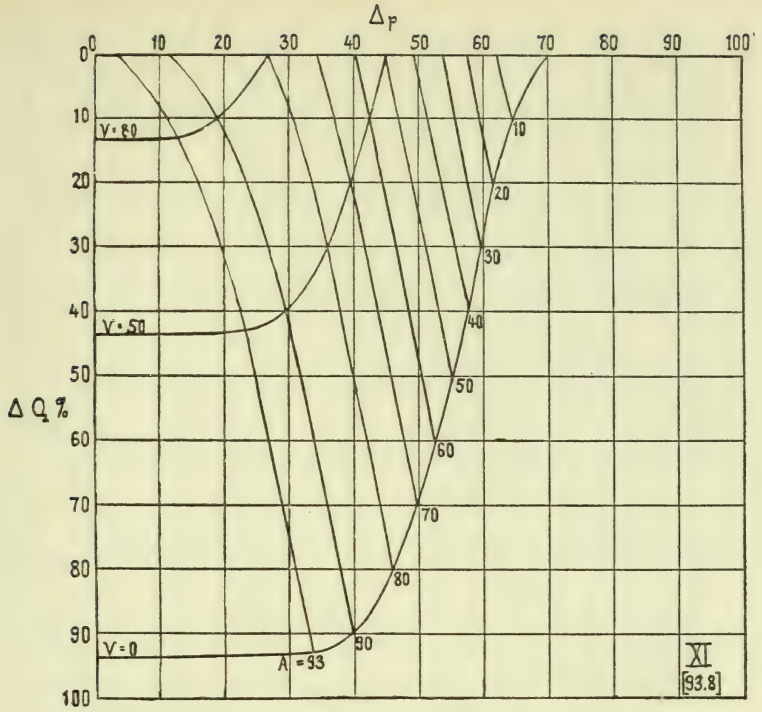

CHART XI pO_2 alveolar air = 70 mm.; pH_a = 7.45.

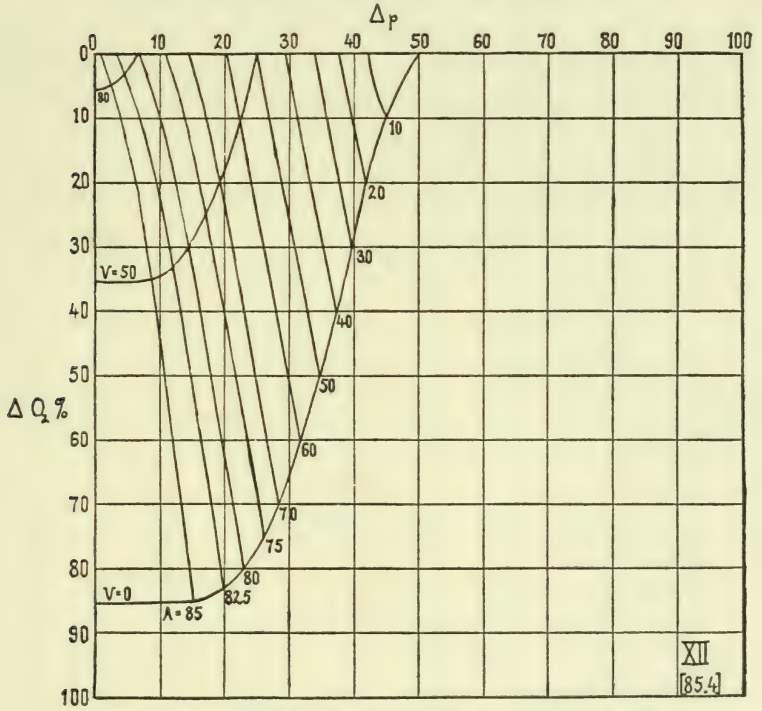

CHART XII. pO_2 alveolar air = 50 mm.; pH_a = 7.45.

Fig. 174 *and Fig.* 175

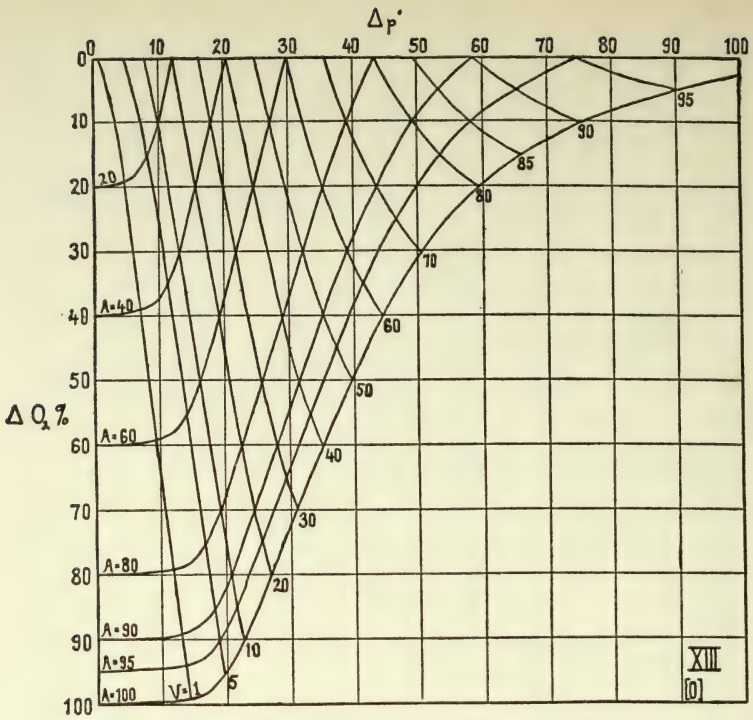

CHART XIII. pO_2 tissues = 0 mm.; pH_s = 7.45.

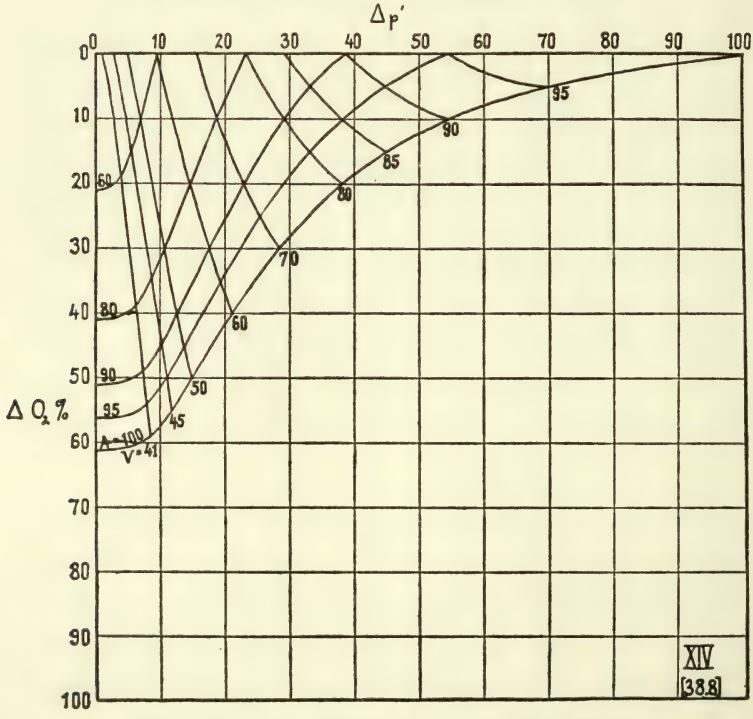

CHART XIV. pO_2 tissues = 20 mm.; pH_s = 7.45.

Fig. 176 *and Fig.* 177

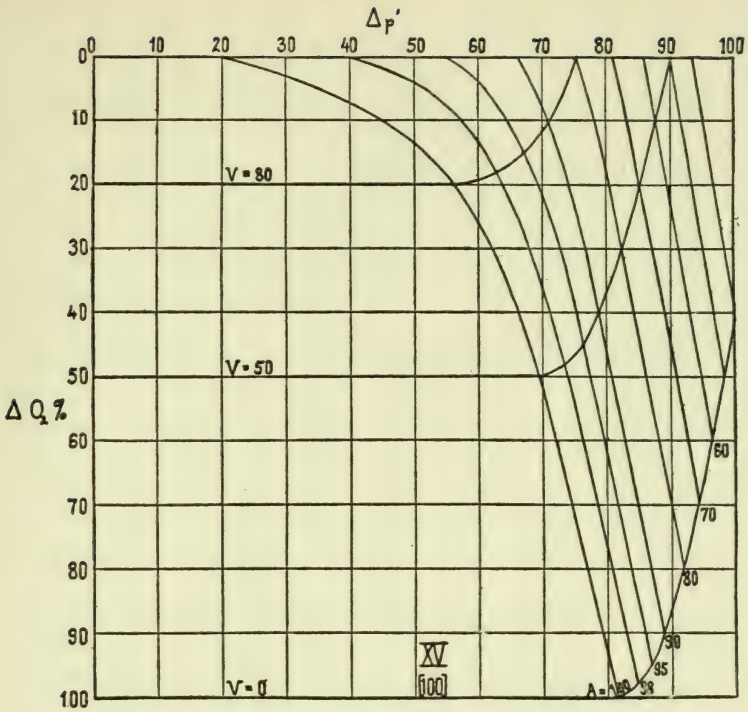

CHART XV. pO_2 alveolar air = 110 mm.; pH_a = 7.65.

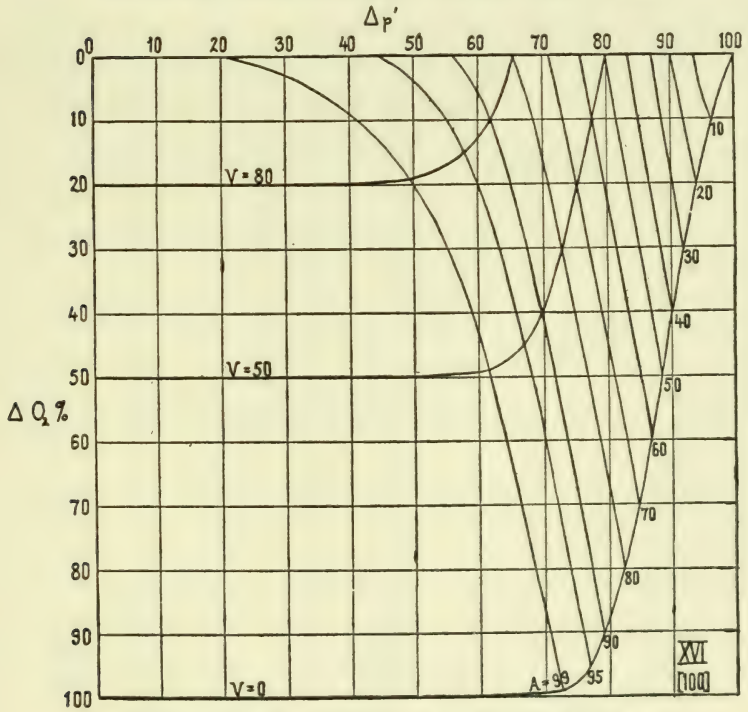

CHART XVI. pO_2 alveolar air = 100 mm.; pH_a = 7.65.

Fig. 178 *and Fig.* 179

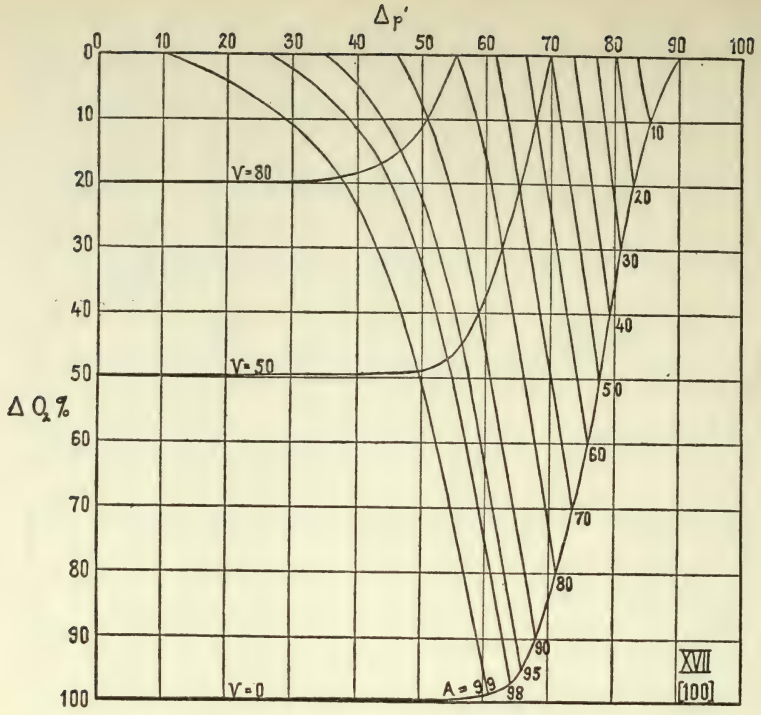

CHART XVII. pO_2 alveolar air = 90 mm.; pH_a = 7.65.

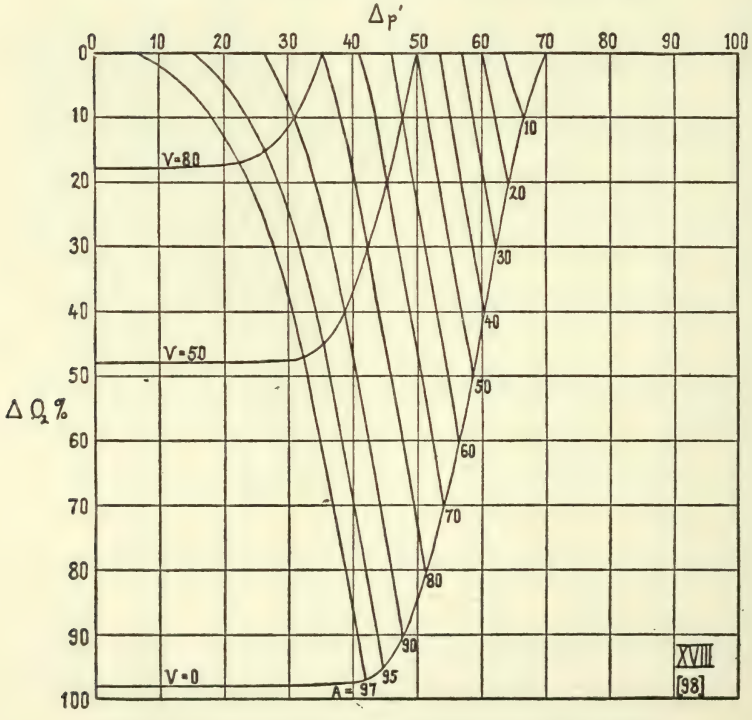

CHART XVIII. pO_2 alveolar air = 70 mm.; pH_a = 7.65.

Fig. 180 *and Fig.* 181

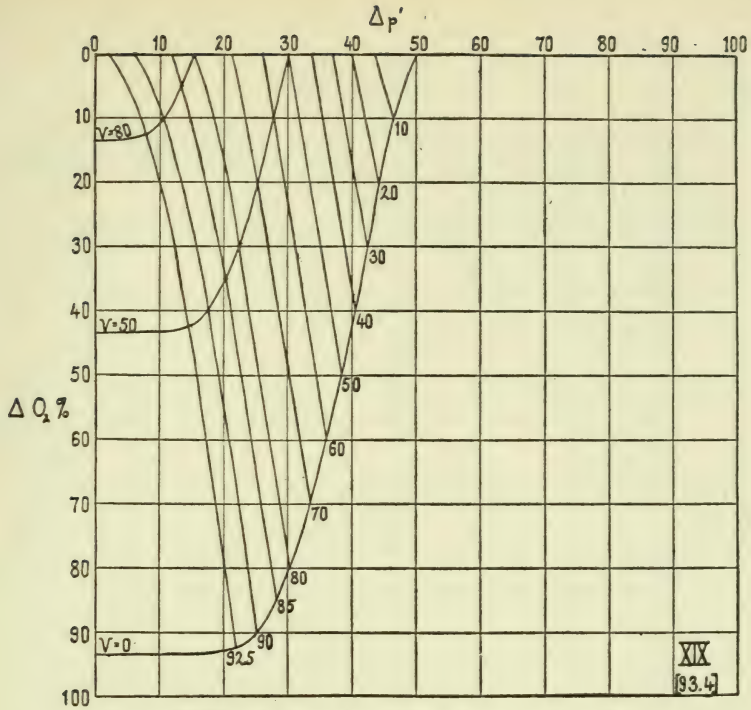

CHART XIX. pO_2 alveolar air = 50 mm.; pH_e = 7.65.

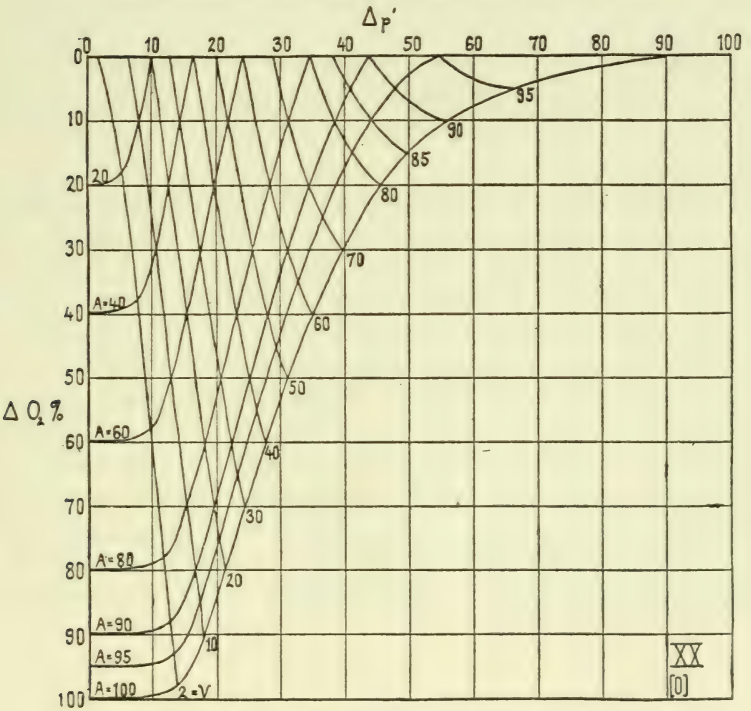

CHART XX. pO_2 tissues = 0 mm.; pH_e = 7.65.

Fig. 182 *and Fig.* 183

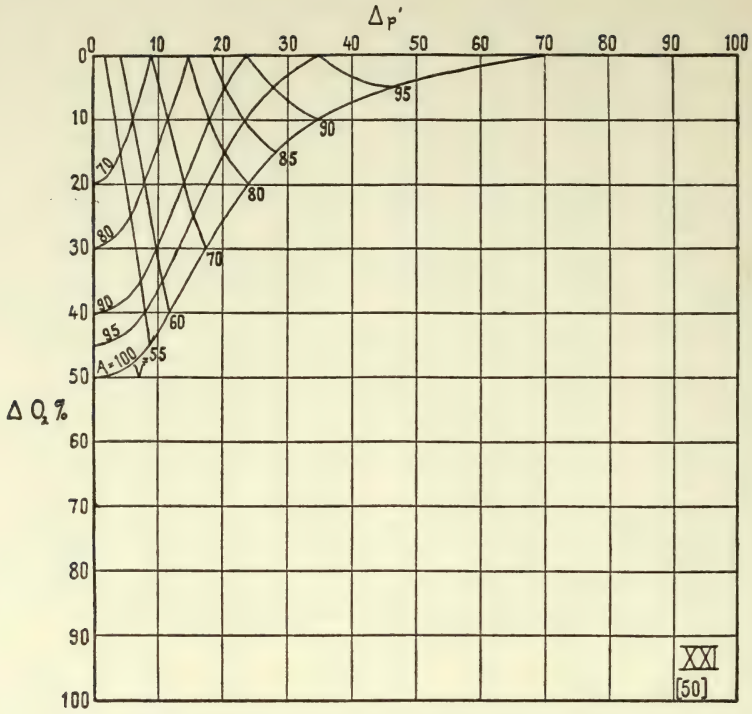

CHART XXI pO_2 tissues = 20 mm.; pH_s = 7.65.

Fig. 184

It will be noticed that, since ΔO_2 per cent is determined by the ordinates of the coördinate system, the set of arterial lines necessarily determines the set of venous lines on the same chart, and *vice versa*. The lines denoting specific hemoglobin flow, SHF, could have been drawn on these charts in accordance with equation 2.

An example of the method of using the charts is as follows: Given pO_2 alveolar air = 95, pH_s = 7.35, A per cent = 95, and V per cent = 65; to find $\Delta p'$ and SHF by double interpolation. First, reference is made to chart II (pO_2 = 100 and pH_s = 7.25), and here the intersection of the line ΔO_2 per cent = 30 with the curve A per cent = 95 yields the value $\Delta p'$ = 39. Similarly, readings are made

on three other charts as indicated in the following tabulation:

pH_s	$pO_2 = 100$	$pO_2 = 90$	$pO_2 = 95$
7.25	II : 39	III : 26	32.5
7.45	IX : 51	X : 40	45.5
7.35	45	33	39.0

The required value for $\Delta p'$ is 39 mm.; and SHF $= \frac{(39 \times 10)}{30} = 13.$

In using the charts the variables may apply to the exchanges of a single capillary, of a single organ, or of the pulmonary circulation or the greater circulation as a whole. When the whole circulation is in question MR designates total metabolism, BF the total blood flow and DC the total diffusing capacity of the lesser or greater circulation of the individual.

The preferable method of use of figure 163 and the charts may now be illustrated. Let the partial pressure of oxygen in alveolar air be 110 mm., the value of pH_s for arterial blood 7.45. Then chart VIII is to be taken as the lower left quadrant of figure 163. Next let the hemoglobin of arterial blood be 96 per cent saturated with oxygen, that of venous blood 75 per cent saturated with oxygen. These two values now fix a point on chart VIII which may be found by interpolation between A and V contour lines of that chart or, more conveniently, by interpolation on the ordinate representing ΔO_2 per cent = 21 (96 − 75 = 21) between the contour lines for A = 98 and A = 95. On chart VIII this point has the coördinates ΔO_2 per cent = 21 per cent, $\Delta p' = 53$ mm. This point may now be marked on figure 163.

As before we take the values Hgb = 20 and MR = 210 and construct a rectangle, the angles of which define all the conditions. In this manner we obtain graphically from the measurements of pO_2 in alveolar air, of pH_s in arterial blood, of the oxygen saturations of arterial and ve-

nous bloods, of the metabolic rate, and of the oxygen capacity of the blood, the values of the other six variables: $\Delta p' = 53$, ΔO_2 per cent $= 21$, SHF $= 25$ (or SDC $= 0.040$), ΔO_2 cc. $= 0.042$, BF $= 5.0$, and DC $= 4.0$. All these results are represented by the dotted rectangle of figure 163.

Murray and Morgan continue the discussion as follows:[126] "If it were possible to determine the oxygen pressure of the tissues, and if this pressure were uniform, the original experimental determinations mentioned above (with a value of pO_2 tissues substituted for pO_2 alveolar air) could be applied to the greater circulation. As it is, the best that can be done in practice is to assume some standard arbitrary value for pO_2 tissues. Thus relative values can be obtained for the other factors which might be of interest. For instance, to continue with the special case just discussed, assuming pO_2 tissues $= 0$, and neglecting for the moment the small difference in pH_s between arterial and venous bloods, one refers to chart XIII. Then, utilizing values already stated above, one finds for the tissues:

(13) $\Delta p' = 51$ mm.,

(14) SHF $= 24$,

(15) DC $= 4.1$.

Other variables, represented by the left portion of the rectangle are identical for lungs and tissues in any given steady state. The only difference between the lung and tissue rectangle lies in the position of the line forming the right side of each rectangle.

"If the oxygen pressure of the tissues were uniform, regardless of its actual value, the whole process of oxygen exchange and transport in lungs and tissues together could be described fairly accurately as the operation of a system with seven degrees of freedom. It is interesting to observe that when the conditions prevailing in the lung have been defined, all but one of the theoretically inde-

[126] Murray and Morgan, *loc. cit.*, p. 438.

pendent variables are approximately determined for the tissues as a whole.

"Although forming no essential part of the present paper, it may be pointed out that the determination of the variables already mentioned leads to certain other factors which amplify the general description of the circulation. For example, if BF be multiplied by the systolic blood pressure, a good estimate of the effective mechanical work of the heart is obtained. Similarly, BF divided by the pulse rate yields a value for the volume output of each ventricle per beat. The blood flow, the blood pressure, and the viscosity of blood, taken together can lead to valuable information concerning the peripheral vessels and circulation."

Murray and Morgan have carefully pointed out the sources of error which are involved in the use of figure 163. One of these, abnormal variation in the relation between values of pH_s and the affinity of hemoglobin for oxygen has been mentioned. A second is due to neglecting the small amount of free oxygen in calculating ΔO_2 per cent. This is in general a source of but slight inconsistency in the results; it is a customary approximation which has often been used in this book. Another source of error is due to the fact that the values of pH_s and pH_c do not remain constant during the respiratory cycle. But, if the values of pH_s for arterial blood be taken in the case of lung diffusion and the values of pH_s for venous blood be taken in the case of tissue diffusion very small errors are involved. This may be seen from inspection of figures 42-146 and of the form of the cycle on figure 148. These conditions depend upon the rapidity with which carbonic acid diffuses, a fact which has been explained in Chapter VII. The assumptions of thorough mixing and of very rapid chemical reaction within the blood itself are other possible sources of error.

We may conclude this discussion with a final quotation from the paper of Murray and Morgan:[127]

[127] Murray and Morgan, *loc. cit.*, p. 441.

"The last question to be raised in respect to the underlying assumptions in our calculations concerns the extension of results, based on the theoretical properties of blood in a given capillary, to cover the lung as a whole or the tissues as a whole. The problem of the tissues is beset with difficulties. In the first place the oxygen pressure of the tissues cannot be accurately determined. Secondly, the oxygen pressure in the tissues along a capillary is probably not uniform, but higher at the end which receives the arterial blood. Thirdly, the mixed venous blood, derived from various organs in which pO_2 may be very different, offers scant information in respect to the precise prevailing conditions. However, marked variations, characteristic of the tissue circulation as a whole or of a significant portion of it, will be reflected in the composition of the mixed venous blood. Thus comparative data may be obtained, and for this purpose it may be useful to select the arbitrary value $pO_2 = 0$ as a reference standard for the oxygen pressure of the tissues.

"In contrast to the diversity of conditions presented by the rest of the body, the lung offers a condition of relative uniformity. The oxygen pressure of the alveolar air can be obtained with considerable accuracy, and this pressure is presumably uniform along the length of a capillary. In the lung there is practically only one case which cannot be accurately described from data on the mixed arterial blood; namely, a condition of *partial* stagnation. After a quantity of blood has nearly reached equilibrium with alveolar air, it may travel through any additional length of capillary without further detectable change in composition. If a considerable quantity of such blood mixes with blood which is not in equilibrium with alveolar air, the mixed blood will not reveal the existence of the superfluous length of capillary through which the first portion has passed. The values of $\Delta p'$, as determined by the data on such mixed arterial blood, will be too high, and consequently the values of the diffusing capacity will be too

low. The superfluous capillary area will not be discovered unless other signs or symptoms suggest its existence."

The results which may be obtained by the use of figure 163 and the auxiliary charts may be demonstrated by taking account of observations of A.V.B. at rest and work. We have from experimental measurements the following data collected in table 29. These yield, by means of the graphical method of Murray and Morgan, the results which are assembled in table 30.

TABLE 29.

A.V.B.

	Rest	Work
pO_2, alveolar air, mm.	110	120
pH_s, arterial blood	7.425	7.351
pH_s, venous blood	7.399	7.278
HbO_2, arterial blood, volumes per cent	97	96.8
HbO_2, venous blood, volumes per cent	74	50.4
ΔO_2 per cent	23	46.4
Hgb, volumes per cent	20	22
MR	250	1,750

TABLE 30.

A.V.B.

		Rest		Work	
Both circulations	BF	5.4		17.3	
	ΔO_2 cc.	0.046			
Pulmonary circulation	$\Delta p'$	50		66	
	DC	5		27	
	SDC	0.046		0.07	
	SHF	21.7		14.3	
pO_2, tissues, mm.		0	20	0	20
Greater circulation	$\Delta p'$	53	32	46	24
	DC	4.7	7.8	38	73
	SDC	0.043	0.072	0.1	0.192
	SHF	23.2	13.9	10	5.2

Too much importance should not be ascribed to the absolute values of these estimates because, unfortunately,

the partial pressure of oxygen in the alveolar air during work was not accurately measured. In the greater circulation values of 0 and 20 mm. for oxygen pressure are assumed in order to make possible the calculations. In spite of such defects, these results will serve as a concrete example of the nature of the relations which have been discussed in the present chapter, and which will later find an application in the comparative studies to which we must now devote our attention.

Comparative studies are indeed inevitable if further progress is to be made beyond the point to which we have now attained, for the first part of our program has been completed.

We have now at our disposal a quantitative description of the components of the physico-chemical system of the blood of vertebrates. To this has been added a description of the system as a whole, then a description of the respiratory cycle of the blood, and finally a description of the necessary relations between this cycle and other physiological activities of the body. As it has been repeatedly stated, these descriptions are all of a roughly approximate character and not only so because of the unavoidable errors of experimental measurements but also because we have chosen to exclude from our treatment of the blood all variables which are not of the first importance. Such approximations I believe to be not a disadvantage but an advantage, or rather a sheer necessity, for without them the first synthetic treatment of the phenomena would be impossible. It is, however, important that all known factors above a certain order of magnitude should be included in the description and this, I hope, has been done. Needless to say, it is only in this sense that the present treatment may be considered complete and there is every reason to expect that new discoveries will lead to additions and modifications.

Thus far we have but incidentally considered the variations in blood, in its respiratory cycle and in its relations to other parts of the body which may be observed when

the physiological state of the individual varies, or when disease leads to more profound modifications of the organism, or when different species of animals are compared. But we are now prepared to undertake such comparative studies in a manner which has not hitherto been followed in biology. With the help of the cumbersome but powerful nomographic methods which have been set forth in the last three chapters, we may proceed to a complete quantitative comparison of the physico-chemical system as a whole and of its respiratory function in rest and work, in health and disease, and in different species. Also, since the components of the system are always the same, in accordance with our simplifying assumptions, we may make all these comparisons at once and thus, as it were, set up a comparative description possessing three dimensions.

CHAPTER IX

WORK

MUSCULAR activity, when sufficiently intense to cause a large increase of metabolism, is accompanied by changes in the composition of the blood. These changes are, however, far from regular. Therefore, they must depend on other factors beside the metabolism.

In general the blood drawn during heavy exercise, when a steady state has been established, contains a rela-

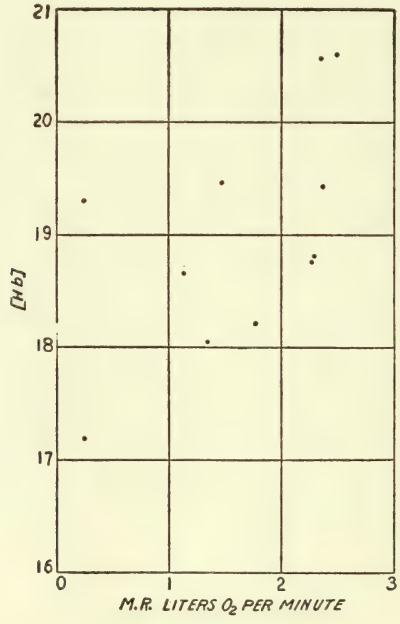

Fig. 185

Oxygen Capacity and Metabolic Rate

tively larger volume of cells and smaller volume of plasma than that drawn from the same individual during rest. The data for D.B.D., shown as points on figure 185, give a fair representation of such variations in volume of the cell phase. On this figure, hemoglobin concentration is plotted against metabolic rate. Under the circumstances, the cells undergo no sensible changes in composition except those due to the usual reversible exchanges with plasma, and the ordinates may, therefore, be taken as a measure of cell volume or of the "red count." The data fall very irregularly about a straight line and it is obvious that the two variables may be positively correlated. If we correct for independent fluctuations in the composition of blood the correlation is more marked. On figure 186 the

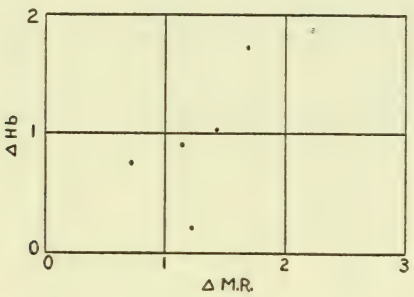

Fig. 186

Variation of Oxygen Capacity with Variation of Metabolic Rate

variation of hemoglobin concentration of blood is plotted against variation of metabolic rate for all cases in which we have made measurements on the same day, of the same blood, during rest and during work. These observations include two separate studies of each of three normal men. It will be seen that in every instance cell volume increases with the metabolic rate and that a rough approximation to proportionality of the two variables may be recognized.

Although the composition of the cells is, under these

circumstances, unchanged, that of the plasma is subject to variation. In general, work seems to be accompanied by a loss of water from the plasma. This may be illustrated by figure 187 on which are plotted changes in the concentration of serum proteins accompanying changes in metabolic rate for five of the six cases just mentioned. Needless to say, the concentrations of serum proteins and of serum water vary inversely. Here again it is plain to see that the variation in serum water must also be a function of other variables.

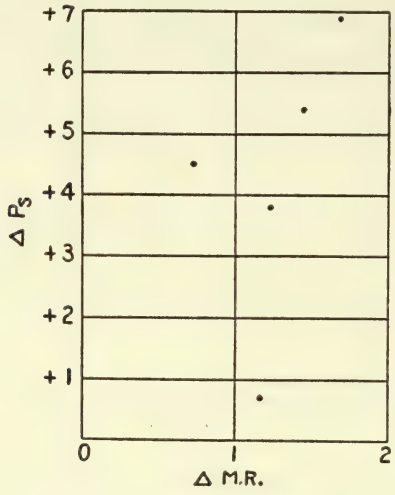

Fig. 187

Variation of Serum Protein with Variation of Metabolic Rate

These changes in the composition of blood during muscular work may perhaps be due to a mobilization of idle red cells and to the passage out of the blood of a fluid having the composition of lymph. In the case of A.V.B., which we are about to study, the changes in cell and plasma volumes, in plasma water and in plasma proteins are such as might be produced by the removal from each

liter of blood of about 50 cc. of lymph and the addition of about 25 cc. of red cells. Such processes are not inconceivable.

A further change in the blood during muscular activity consists in a decrease in its carbonic acid capacity. This may be conveniently, though very roughly, measured by the total carbonic acid concentration of oxygenated blood at a pressure of 40 mm. of carbon dioxide, *i.e.*, by the so-called level of the carbon dioxide dissociation curve. The variation in level of this curve is to be attributed chiefly to the neutralization of lactic acid, although the increase of sodium lactate in the blood is not here, as it is under other circumstances, equivalent to the fall in level. This is partly on account of differences in pH_c, through buffer action of the protein salts of blood, partly through exchanges of other substances, in addition to lactic acid, with the lymph, the muscles, and the other tissues. The facts are illustrated by figure 188. Here the two middle curves represent the mean values, roughly estimated, for a large number of observations on A.V.B., D.B.D., and L.M.H. It may be seen that low degrees of muscular ac-

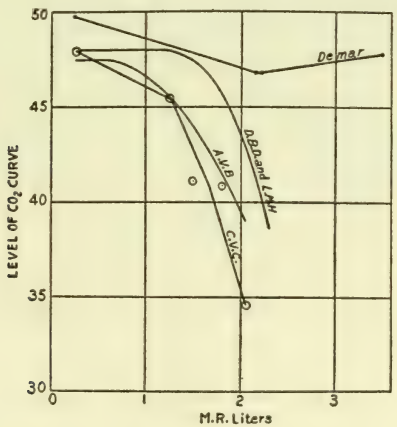

Fig. 188

Blood Carbonic Acid and Metabolic Rate

tivity have little or no effect upon the level of the carbon dioxide dissociation curve, but that with greater activity there is a marked indication of acidosis. Figure 188 also shows the data for two men of about the same size, one of whom, De Mar, is a long distance runner of very superior power, in the best of training, while the other, C.V.C., is a physician who is not in the habit of taking much exercise.

In the case of De Mar, it is evident that there is no effect of exercise upon the level of the curve beyond small fluctuations attributable to other causes than acidosis, such as mobilization of red cells and other similar readjustments. The difference between this case and the other is very large indeed and speaks for itself. It is confirmed by measurements of the lactate content of the bloods of De Mar and of C.V.C. which were made on the same specimens of blood. The data are shown on figure 189. Evidently De Mar, whose blood seems normally to contain a

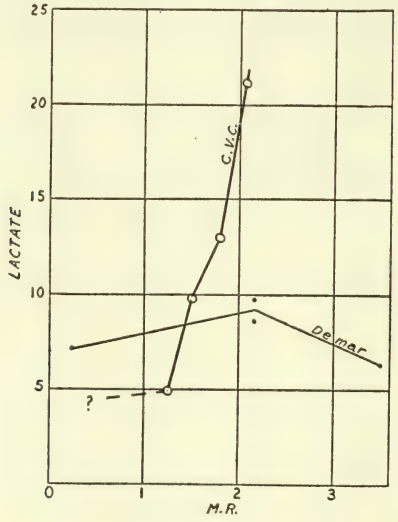

Fig. 189

Blood Lactate and Metabolic Rate

fairly high concentration of lactate, may work continuously at a metabolic level beyond the reach of C.V.C., without the slightest increase of lactic acid in the blood. During a quarter century of experimental work, I have never seen a greater contrast than that illustrated by figure 189.

We may sum up the effect of muscular work upon blood bicarbonate and lactate by the statement that this is for small increases in work inappreciable and for larger increases more and more important, but that the metabolic level at which the effect sets in is very variable in different individuals.

In order to pursue the consideration of the changes in blood which accompany muscular activity, it will now be necessary to study a single case quantitatively and in detail. For this purpose we may make use of the nomogram, figure 190,[128] which describes the blood of A.V.B. when the stationary state has been reached during work involving an oxygen consumption of 1750 cc. per minute. This is about nine times the resting metabolic rate. The work consisted in riding a stationary bicycle. Blood was invariably drawn after a period of not less than fifteen minutes of uniform work and while work was being continued. This nomogram is to be compared with figure 157, Chapter VII, which describes the blood of the same individual at rest on the same day.

[128] Bock, Dill, Hurxthal, Lawrence, Coolidge, Dailey, and Henderson, *Journal of Biological Chemistry,* LXXIII, 749 (1927).

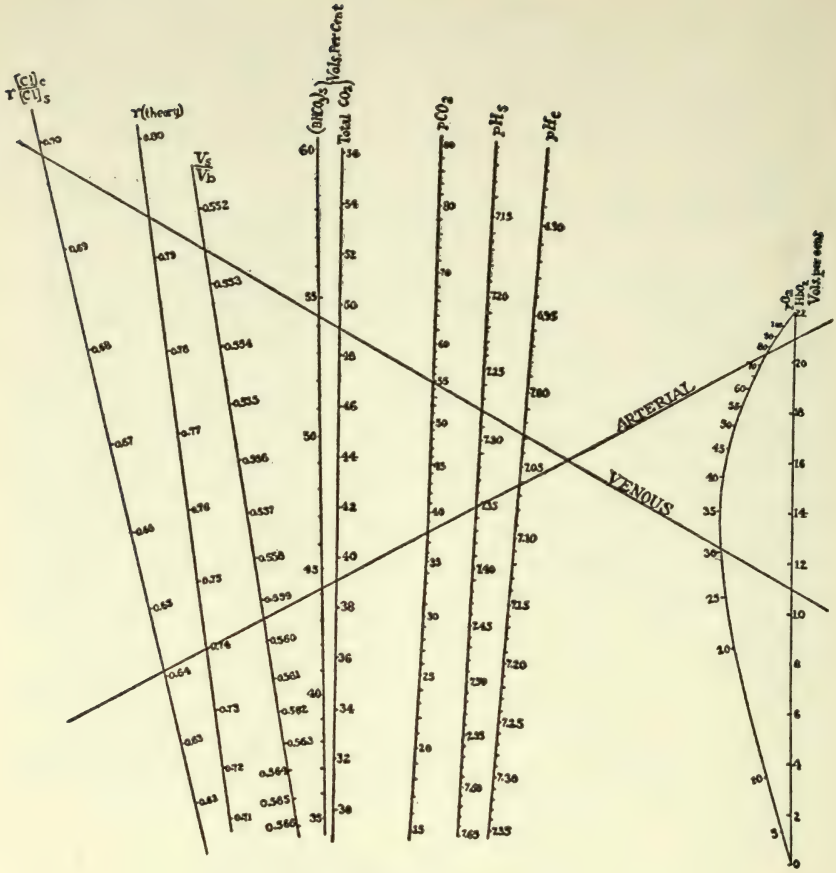

Fig. 190

Blood of A.V.B. at Work

TABLE 31.

Blood of A.V.B. at work.

Concentration of hemoglobin = 9.82 *mM* per liter of blood.
Concentration of serum proteins = 44.8 gm. per liter of blood.
Respiratory quotient = 1.00.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	525	310.5	835.5	517.6	317.9	835.5	−7.4	+7.4	0.0
B *mM* " " "	89.60	58.37	147.97	89.60	58.37	147.97			
X " " " "	13.31	8.83	22.14	13.23	9.09	22.32	−0.08	+0.26	+0.18
Cl " " " "	57.48	21.77	79.25	55.44	23.81	79.25	−2.04	+2.04	0.0
BP " " " "	7.79	22.39	30.18	7.57	18.41	25.98	−0.22	−3.98	−4.20
$BHCO_3$ " " " "	11.02	5.38	16.40	13.36	7.06	20.42	+2.34	+1.68	+4.02
$BHCO_3$, *vol. per cent*	24.69	12.05	36.74	29.94	15.80	45.74	+5.25	+3.75	+9.00
H_2CO_3, *mM per l. blood*	0.65	0.38	1.03	0.92	0.56	1.48	+0.27	+0.18	+0.45
H_2CO_3, *vol. per cent*	1.45	0.86	2.31	2.06	1.26	3.32	+0.61	+0.40	+1.01
Total CO_2, *mM per l. blood* ..	11.67	5.76	17.43	14.28	7.62	21.90	+2.61	+1.86	+4.47
Total CO_2, *vol. per cent*	26.14	12.91	39.05	32.00	17.06	49.06	+5.86	+4.15	+10.01
Free O_2, *mM per l. blood*			0.09			0.04			−0.05
Free O_2, *vol. per cent*			0.19			0.08			−0.11
Combined O_2, *mM per l. blood*			9.33			4.91			−4.42
Combined O_2, *vol. per cent* ..			20.90			11.00			−9.90
Total O_2, *mM per l. blood* ...			9.42			4.95			−4.47
Total O_2, *vol. per cent*			21.09			11.08			−10.01
pCO_2, *mm. Hg*			38.0			54.8			+16.8
pO_2, *mm. Hg*			75.0			31.0			−44.0
Volume, *cc. per l. blood*	559.5	440.5	1,000.0	552.6	447.4	1,000.0	−6.9	+6.9	
pH	7.351	7.062		7.278	7.027		−0.073	−0.035	
r_{theory}			0.740			0.793			+0.053
r_{HCO3}			0.825			0.859			+0.034
r_{Cl}			0.641			0.699			+0.058

TABLE 32.

Serum of A.V.B. at work.

		Arterial	Venous	Δ	$\frac{100\Delta}{A}$
H_2O	*cc. per l. serum*	938.4	936.7	−1.7	−0.2
B	*mM* " " "	160.1	162.1	+2.0	+1.2
X	" " " "	23.78	23.92	+0.14	+0.6
Cl	" " " "	102.7	100.3	−2.4	−2.3
BP	" " " "	13.92	13.70	−0.22	−1.6
$BHCO_3$	" " " "	19.70	24.18	+4.48	+22.7
H_2CO_3	" " " "	1.16	1.66	+0.50	+43.0
Total CO_2	" " " "	20.86	25.84	+4.98	+23.9

TABLE 33.

Cells of A.V.B. at work.

		Arterial	Venous	Δ	$\frac{100\Delta}{A}$
H_2O	*cc. per l. cells* ...	704.9	710.5	+5.6	−0.8
B	*mM* " " " ...	132.5	130.5	−2.0	−1.5
X	" " " " ...	20.04	20.32	+0.38	+1.4
Cl	" " " " ...	49.42	53.22	+3.80	+7.7
BP	" " " " ...	50.83	41.15	−9.68	−19.0
$BHCO_3$	" " " " ...	12.22	15.78	+3.56	+29.1
H_2CO_3	" " " " ...	0.86	1.25	+0.39	+45.5
Total CO_2	" " " " ...	13.08	17.03	+3.95	+30.2
Combined O_2	" " " " ...	21.18	10.97	−10.21	−48.4

TABLE 34.

Arterial serum of A.V.B. at rest and at work.

		Rest	Work	Δ	$\frac{100\Delta}{\text{Rest}}$
H_2O	*cc. per l. serum*	943.3	938.4	−4.9	−0.5
B	*mM* " " "	154.0	160.1	+6.1	+4.0
X	" " " "	17.00	23.78	+6.78	+40.0
Cl	" " " "	99.32	102.70	+3.38	+3.4
BP	" " " "	13.13	13.92	+0.79	+6.0
$BHCO_3$	" " " "	24.55	19.70	−4.85	−19.8
H_2CO_3	" " " "	1.22	1.16	−0.06	−4.9
Total CO_2	" " " "	25.77	20.86	−4.91	−19.1
pH		7.425	7.351	−0.074	

TABLE 35.

Arterial cells of A.V.B. at rest and at work.

		Rest	Work	Δ	$\frac{100\Delta}{\text{Rest}}$
H_2O	*cc. per l. cells* ...	705.0	704.9	−0.1	0.0
B	*mM* " " " ...	133.75	132.5	−1.25	−0.9
X	" " " " ...	17.73	20.04	+2.31	+13.0
Cl	" " " " ...	45.27	49.42	+4.15	+9.2
BP	" " " " ...	56.50	50.83	−5.67	−10.0
$BHCO_3$	" " " " ...	14.25	12.22	−2.03	−14.2
H_2CO_3	" " " " ...	0.93	0.86	−0.07	−7.5
Total CO_2	" " " " ...	15.18	13.08	−2.10	−13.8
Combined O_2	" " " " ...	21.43	21.18	−0.25	−1.2
pH		7.124	7.062	−0.062	

TABLE 36.

Respiratory changes in rest and work.

	SERUM				CELLS				WHOLE BLOOD			
	I Rest	II Work	II − I	II : I	I Rest	II Work	II − I	II : I	I Rest	II Work	II − I	II : I
H_2O *cc. per l. blood*	−4.0	−7.4	3.4	1.85	+4.0	+7.4	3.4	1.85				
X *mM " " "*	+0.18	−0.08	?	?	−0.18	+0.26	?	?		0.18		
Cl " " " "	−0.87	−2.04	1.17	2.34	+0.87	+2.04	1.17	2.34				
BP " " " "	−0.08	−0.22	0.14	2.75	−1.45	−3.98	2.53	2.74	−1.53	−4.20	2.67	2.74
$BHCO_3$ " " " "	+0.77	+2.34	1.57	3.05	+0.76	+1.68	0.92	2.18	+1.53	+4.02	2.49	2.61
$BHCO_3$, *vol. per cent*	+1.71	+5.25	3.54	3.05	+1.72	+3.75	2.03	2.18	+3.45	+9.00	5.55	2.61
H_2CO_3, *mM per l. blood*	+0.10	+0.27	0.17	2.70	+0.05	+0.18	0.13	3.60	+0.15	+0.45	0.30	2.97
H_2CO_3, *vol. per cent*	+0.22	+0.61	0.39	2.70	+0.12	+0.40	0.28	3.60	+0.34	+1.01	0.67	2.97
Total CO_2, *mM per l. blood*	+0.87	+2.61	1.74	3.00	+0.81	+1.86	1.05	2.30	+1.68	+4.47	2.79	2.65
Total CO_2, *vol. per cent*	+1.93	+5.86	3.93	3.00	+1.84	+4.15	2.31	2.30	+3.77	+10.01	6.24	2.65
Free O_2, *mM per l. blood*									−0.04	−0.04	0.01	1.25
Free O_2, *vol. per cent*									−0.10	−0.11	0.01	1.10
Combined O_2, *mM per l. blood*					−2.01	−4.42	2.41	2.20	−2.01	−4.42	2.41	2.20
Combined O_2, *vol. per cent*					−4.50	−9.90	5.40	2.20	−4.50	−9.90	5.40	2.20
Total O_2, *mM per l. blood*									−2.05	−4.47	2.42	2.18
Total O_2, *vol. per cent*									−4.60	−10.01	5.41	2.18
pCO_2, *mm. Hg*									+5.4	+16.8	11.4	3.11
pO_2, *mm. Hg*									−38.0	−44.0	6.0	1.16
Volume, *cc. per l. blood*	−3.8	−6.9	3.1	1.82	+3.8	+6.9	3.1	1.82				
pH	−0.026	−0.073	−0.047	2.80	−0.008	−0.035	0.027	4.4				
H	$+21.10^{-10}$	$+78.10^{-10}$	$+57.10^{-10}$	3.71	$+14.10^{-10}$	$+75.10^{-10}$	61.10^{-10}	5.36				
r_{theory}									+0.025	+0.053	0.028	2.12
r_{HCO_3}										+0.034		
r_{Cl}									+0.025	+0.058	0.033	2.32

The composition of arterial and of venous blood, of plasma and of cells, the changes of the respiratory cycle, and a comparison with the resting state, are given in tables 31 to 36. Table 31 presents the values of the variables in arterial blood and in mixed venous blood, together with their differences. These differences measure the physiological respiratory cycle. Tables 32 and 33 include the corresponding values of the more important variables for serum and for cells. In tables 34 and 35 the arterial serum and arterial cells of the working and resting states are compared. In table 36 the respiratory changes, *i.e.*, differences between arterial and venous conditions for serum, cells, and whole blood, at work and at rest, are compared.

On the whole these tables call for no comment. It is necessary, however, in order to appreciate their significance, to take account of certain facts. First, as the nomogram and the tables show, the differences between arterial and venous bloods at work are much greater than the differences at rest. Secondly, the respiratory quotient has risen from 0.80 at rest to 1.00 at work. The first of these facts is chiefly due to the great increase in the coefficient of utilization of oxygen and to the even greater increase in the amount of carbonic acid taken up by the blood in a single passage through a tissue capillary and liberated in the lung. To the increased magnitude of the respiratory exchanges the 10 per cent increase in hemoglobin content of the blood also contributes. The change in respiratory quotient is presumably dependent upon the utilization of carbohydrate in the muscle, in accordance with Hill's views.[129]

This value of 1.00 for the respiratory quotient is within the normal range, but somewhat above the mean of our experience. The facts are best illustrated by two unusually successful experiments on D.B.D. in which the subject worked at a uniform rate corresponding to a con-

[129] A. V. Hill, *Muscular Activity,* Baltimore, 1926.

sumption of oxygen of about 2000 cc. per minute (1995 cc. on February 21; 1965 cc. on March 30) during the period following the tenth minute, for about one hour. The data are plotted on figure 191. On February 21 the mean value

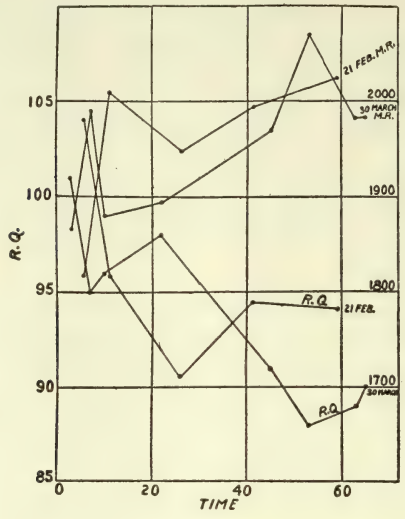

Fig. 191

Respiratory Quotient and Metabolic Rate

of the respiratory quotient for the period 10 to 60 minutes was about 0.94, on March 30 for the period 10 to 65 minutes about 0.93.

On February 21 the level of the carbon dioxide dissociation curve was at 15 minutes 44.8 and at 60 minutes 45.7; on March 30 at 18 minutes 42.9 and at 75 minutes 45.6. It may be deduced from these observations that approximately a stationary state existed after the first 10 minutes and, in particular, that carbonic acid was not then being lost from the body through the action of acid. This is confirmed by the fact that in the first experiment the blood contained 550 mg. lactic acid per liter at 15 minutes and 430 mg. at 60 minutes. In the second experiment

there must have been, in fact, a slight retention of carbonic acid, and we may regard the mean respiratory quotient of the two experiments as 0.94. The respiratory quotient of this subject at rest is not more than 0.80 and the facts indicate a value of 0.96 for the respiratory quotient of the active muscle.

We have also at our disposal data bearing upon this question from a large number of experiments on four different subjects. In these experiments the metabolic rate, which varied considerably from case to case, corresponded on the average to an oxygen consumption of about 1900 cc. per minute. In the following table the values of the respiratory quotient in all these cases at regular 10-minute intervals from the beginning of work are given.

TABLE 37.

	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
	1.04	1.03	.94	.88	.94	.94
	.94	1.00	.94	.94	1.06	
	.93	.93	.86	.95		
	.96	.90	.87	.89		
	.94	.97	.98	1.03		
	.91	.97	.90			
	.90	.89	.91			
	.86	1.00				
	1.02	.91				
	.99	.88				
	.99	.86				
	.94					
	.92					
Mean	.949	.94	.914	.938	1.00	.94

Mean of all observations, .94
Respiratory quotient of muscular activity, .96

In many of these experiments the respiratory quotient of the resting state was measured and found invariably much lower than in work, with an average of 0.80. It is possible, therefore, to estimate for all these cases the respiratory quotient of muscular activity as about 0.96.

It will be seen that the values of the respiratory quotients, although invariably far above those of the resting state, are variable. The data just cited included those of one experiment on De Mar in which during the period from 10 minutes to the end of the experiment at 24 minutes the respiratory quotient remained constantly a little below 0.90. There are also two values, which balance these, in which the respiratory quotient was high, probably on account of fatigue at the end of the experiment (1.03 at 40 minutes and 1.06 at 50 minutes).

In some of these experiments the subject had recently eaten, in others he had fasted for 15 hours. Differences in this respect seem to be without effect upon the respiratory quotient of the working condition and in these cases are certainly without large effect.

From all these experiments the conclusion is unescapable that, during the stationary state of work, the respiratory quotient is greatly increased. We have, however, noted a tendency in very prolonged work without excessive fatigue to a slight fall in the value of the respiratory quotient. Altogether it may be seen that the observations are far from uniform. This is to be expected, since many physiological variables are involved. But there can be no doubt that all our observations are consistent with the hypothesis that muscular work of moderate duration is characterized by an intrinsic respiratory quotient of 1.00.

The changes in blood, which accompany muscular activity, result in slight modifications of the mechanism of the transport of carbon dioxide. These depend upon the lowering of the level of the carbon dioxide dissociation curve and upon the increased acidity of the blood, slight in the arterial blood, much greater in the venous blood, and considerable for the average composition throughout the cycle. These changes also produce a certain modification of the transport of oxygen.

It will be profitable to consider the transport of carbonic acid quantitatively and in detail. First, since dur-

ing work the level of the carbon dioxide curve is considerably lowered without much change in slope, it follows that, other things being equal, a given change in total carbonic acid is accompanied by an increased variation in hydrogen ion concentration. But the magnitude of such effects is small and, together with others of the same order, such as those due to the differences in heterogeneous equilibrium, negligible. The relatively important differences between rest and work can all be interpreted in terms of the oxygen effect and of the true buffer action of the proteins.

To this end we may consider table 38 which includes in column one data from table 15, Chapter VII; in column two data from table 19, Chapter VII; and in column three data from table 31 of the present chapter. In Chapter VII it was deduced from the data shown on table 38, column one, and the large nomogram, that for the conditions in

TABLE 38.

Respiratory variation in blood of A.V.B.

	Earlier estimate (light work)	Rest	Work
ΔHbO_2	−2.70	−2.01	−4.42
ΔpH_s	−0.029	−0.026	−0.073
ΔpH_c	−0.009	−0.008	−0.035
ΔBP_s	−0.11	−0.08	−0.22
ΔBP_c	−1.97	−1.45	−3.98
ΔBP_B	−2.08	−1.53	−4.20
$\Delta(BHCO_3)_B$	+2.08	+1.53	+4.02
ΔBX			+0.18
Total (Hb)	8.80	8.93	9.82

question the oxygen effect accounted for 1.71 millimoles, true buffer action of hemoglobin for 0.26 millimole, and buffer action of the serum proteins for 0.11 millimole of the total change, 2.08 millimoles in $(BP)_B$. It will be remembered that this is equal to the total change in $(BHCO_3)_B$.

These values are reproduced in column one of table 39 and from them, with the help of the theory discussed in Chapter IV, are calculated the values of the second and third columns of this table. The total changes in BP_B thus

TABLE 39.

	Earlier estimate		Rest	Work
ΔBP_c oxygen effect	−1.71	calculated	−1.27	−2.80
ΔBP_c buffer effect	−0.26		−0.23	−1.11
ΔBP_s buffer effect	−0.11		−0.08	−0.22
Calculated ΔBP_B			−1.58	−4.13
Observed ΔBP_B			−1.53	−4.20
Observed $\Delta(BHCO_3)_B$			+1.53	+4.02

calculated correspond closely with the observed values of $\Delta(BP)_B$ and $\Delta(BHCO_3)_B$ of the second and third columns of table 39. In table 40 the relative importance of the oxygen effect, of total buffer action and of buffer action of cell and serum proteins separately are indicated.

TABLE 40.

Relative importance of the factors.

	Earlier estimate	Rest	Work
Oxygen effect	82	80	68
Total buffer action	18	20	32
Hemoglobin buffer action	13	15	27
Serum protein buffer action	5	5	5

In table 40 the values of the first and second columns are approximately equal, but the third column shows that during work the relative importance of true buffer action and oxygen effect is much changed. This depends upon the change in the respiratory quotient, for the oxygen effect is proportional to the change in combined oxygen, while the true buffer action varies directly with change in total carbon dioxide and inversely with change

in combined oxygen. Accordingly, there must be conditions where change in carbonic acid and change in oxygen content just balance, so that the value of pH_c remains unchanged and all true buffer action of hemoglobin is suppressed. Though slightly variable, these conditions are, as we have seen, characterized by a respiratory quotient of approximately 0.7 and in fact the pH_c scale of the nomogram falls very close to the line of R.Q. = 0.70. In other words, one millimole increase in the total oxygen of the blood has in respect of the values of BP_c and pH_c approximately the same effect as 0.7 millimole increase in total carbonic acid. Changes in the conditions within the cell are of course transmitted to the plasma and affect the values of pH_s, with the result that differences in hydrogen ion concentration between arterial and venous bloods as a whole become greater as the respiratory quotient increases from the value of approximately 0.7. The process also continues as the value of the respiratory quotient falls below the value 0.7. Under these circumstances the value of ΔpH_c for the respiratory cycle, after passing through 0, changes its sign, and for values of the respiratory quotient below 0.7 it becomes negative.

On figure 192 there are given the relations of the respiratory quotient to the difference in values of pH_c and of pH_s for arterial and venous bloods when there is a constant coefficient of utilization of oxygen, for blood of average composition. It must be understood that this figure represents a rough approximation.

As the relative importance of the oxygen effect upon the transport of carbonic acid diminishes, the relative importance of the effect of carbonic acid upon the transport of oxygen does not decrease, but in general increases. This depends upon the fact already noted that the important variable in these variations is the value of pH_c. In the resting state this effect is almost negligible and is equivalent to one-tenth of the oxygen transported. With increase in respiratory quotient and in coefficient of uti-

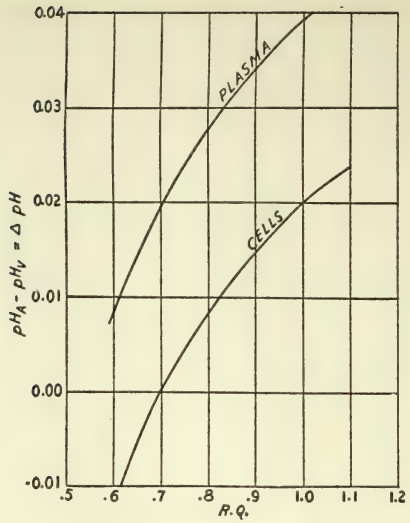

Fig. 192

Respiratory Quotient and Variation of Hydrogen Ion Concentration

lization the value becomes greater. We may read from the nomogram the following pairs of values:

TABLE 41.

	pO_2	HbO_2	Total CO_2
1	31	12.80	39.05
2	31	11.08	49.06

The first row of numbers gives the values of three variables in blood which has the oxygen pressure of venous blood but the total carbonic acid content of arterial blood. The second row gives the values for venous blood. It will be seen that there is a difference of 1.7 volumes per cent between the oxygen contents of the blood in these two states. This may be regarded as the measure of the carbonic acid effect upon oxygen transport for the condition of work described by the nomogram. Since the oxygen

utilization (arterial total oxygen minus venous total oxygen) is 10 volumes per cent, it is evident that the carbonic acid effect is here responsible for about one-sixth of the oxygen carried by the blood. Utilization is, under these circumstances, double that of the resting state. Hence the absolute measure of the effect of carbonic acid upon the oxygen transport is three times as great as in the resting state.

We have seen that for these conditions the oxygen effect amounts to 2.8 mm. or 6.3 volumes per cent. This is, accordingly, nearly four times as great as the carbonic acid effect.

Since, in calculating the carbonic acid effect, an arbitrary definition is involved, it may be more convenient for certain purposes to measure this effect by means of the difference in oxygen content between venous blood, on the one hand, and blood having the oxygen pressure of venous blood and the carbon dioxide pressure of arterial blood, on the other hand. In this case the estimate will yield somewhat different values for the effect.

In considering the oxygen effect, we are able to calculate the changes in ionization of certain acid radicals of the protein molecule, to contrast these with the changes in the hemoglobin molecule, and so to reach a definite conclusion. But we have no such means of defining the carbonic acid effect. Therefore, it is necessary in this case to adopt an arbitrary definition and, if a complete description is desired, to make use of a complete nomogram. The facts may be summed up as follows: The oxygen effect is always large and this finds its expression in the wide divergence in the variations in carbonic acid of the physiological respiratory cycle from the ordinary carbon dioxide dissociation curves. The carbonic acid effect is small and the curve of the physiological respiratory cycle for oxygen closely parallels the ordinary oxygen dissociation curve. Both effects vary with the coefficient of utilization of oxygen and with variation in the respiratory quotient. When this is high, as in work, the transport of oxy-

gen may be sensibly facilitated, while, at the same time, true buffer action becomes relatively more important in the transport of carbonic acid. Finally, when we speak of the carbonic acid effect we are obliged to adopt an arbitrary definition in order to have any measure at all, but when we refer to the oxygen effect, we use an unambiguous term and have in mind a quantity which may be easily measured. This statement is not quite precise, since the buffer action of oxyhemoglobin is slightly greater than that of reduced hemoglobin. Therefore, if, starting with arterial blood, we wish to calculate the precise buffer action of hemoglobin during the process by which the blood becomes venous, we must make an intricate calculation of the rate of change of oxygenation as a function of the rate of change of pH_c, before we can begin to make this estimate. Practically, however, this is of no importance, for the mean buffer value of the mixture of oxyhemoglobin and reduced hemoglobin of arterial and venous blood may be used without sensible error.

The difficulty which here confronts us is only an apparent one, for the facts are given by the nomogram and the theoretical discussions of the preceding chapters are sufficient for their interpretation.

The use of measurements of the level of the carbon dioxide dissociation curves as an index of the acid-base composition of the blood is a possible source of misunderstanding and, for many purposes, a better measure is given by the sum of $(BP)_B + (BHCO_3)_B$, which represents the available base of the blood. A still better measure is afforded by the quantity $\frac{(BP)_B + (BHCO_3)_B}{(H_2O)_B}$. It will, therefore, be useful to take account of the nomogram as a means of defining the values of these quantities in the case of the blood of A.V.B. at work and thus to supplement what has been said above. The relevant facts are as follows:

TABLE 42.

Arterial blood of A.V.B.

	$(H_2O)_B$ cc. per l.	$(BP)_B$ *mM* per l. blood	$(BHCO_3)_B$ *mM* per l. blood	$(BP)_B + (BHCO_3)_B$ *mM* per l. blood
Work	835.5	30.18	16.40	46.58
Rest	848.0	30.48	20.43	50.91
		$(BP)_B$ *mM* per l. H_2O	$(BHCO_3)_B$ *mM* per l. H_2O	$(BP)_B + (BHCO_3)_B$ *mM* per l. H_2O
Work		36.1	19.6	55.7
Rest		35.9	24.1	60.0

For work the level of the carbon dioxide dissociation curve is 17.86 millimoles, for rest 20.32 millimoles, and the difference of these two values is 2.5 millimoles. But the difference of the two values of the quantity $(BP)_B + (BHCO_3)_B$ is 4.3 millimoles and of the two values of the quantity $\frac{(BP)_B + (BHCO_3)_B}{(H_2O)_B}$ it is also in this case 4.3 millimoles. Measurements of the lactic acid content were, unfortunately, not made in this blood. But all our experience indicates that the value of this quantity at this level of muscular activity is much more nearly 4.3 millimoles than 2.5 millimoles. The disparity between the results of the two methods of estimating the change in the composition of blood depends upon the fact that for a pressure of 40 mm. of carbon dioxide there are considerable variations in the values of pH_c in different specimens of blood. For example, we have in the working and resting bloods of A.V.B., when fully oxygenated at a pressure of 40 millimeters of carbon dioxide, the pH_c values 7.040 and 7.113 respectively. Therefore, in the working blood relatively less base is combined with proteins and relatively more with carbonic acid. On the other hand, the sum $(BP)_B + (BHCO_3)_B$ is approximately constant.

The sum $(B)_B + (Cl)_B + (X)_B + (BHCO_3)_B$ is a rough measure of the total concentration of the blood, and the sum of the concentrations of these substances per liter of water is a more accurate measure of this quantity. For

the blood of A.V.B. in work and in rest we have the following values:

TABLE 43.

Arterial blood of A.V.B.

	$(B)_B$	$(Cl)_B$	$(X)_B$	$(BHCO_3)_B$	Σ
	mM per liter blood.				
Work	148.0	79.3	22.1	16.4	265.8
Rest	145.9	77.7	17.3	20.4	261.3
	mM per liter water.				
Work	177.1	94.8	26.5	19.6	318.0
Rest	172.1	91.6	20.4	24.1	308.2

It is obvious that the two sums, 318.0 millimoles and 308.0 millimoles per liter water must fall appreciably short of the total concentration of dissolved material in the two samples of blood. But, as a measure of the change of concentration of the blood of A.V.B., their difference can hardly be very inexact, except through experimental error. Accordingly, it appears that exercise has resulted in an increase of about three per cent in the concentration of the blood solution. A similar calculation based upon the estimates of the composition of the serum gives a value of about four per cent for the increase of concentration. In view of uncertainties regarding the values of r_{Cl} and the large effect of this value upon the present estimate, and in view also of the large number of factors involved in the estimates, the agreement is satisfactory.

Such an increase in concentration is to be expected, for, during active metabolism, the muscle solution must attain to a very high concentration, the effects of which cannot fail to be transmitted to other parts of the body. No doubt lactic acid has a large share in this effect, but all components of the system must be at least indirectly involved, with results, so far as blood is concerned, which are at present theoretically incalculable. Added to these effects there may be concentration of the body fluids resulting from the evaporation of water.

We have before us estimates of these changes based upon measurement. It will be seen that the concentration of blood chloride has increased three millimoles per liter, that of salts of other acids, including lactic acid, six millimoles per liter, or a total of nine millimoles per liter for all acid. Meanwhile, total base has increased nine millimoles per liter and, as we have seen, $(BP)_B + (BHCO_3)_B$ has decreased four millimoles per liter, in accordance with the relation $\Delta(Cl)_B + \Delta(X)_B = \Delta(B)_B - \Delta(BP)_B - \Delta(BHCO_3)_B$.

These remarkable changes, especially in the concentration of chloride, which takes no direct part in the chemical reactions of metabolism and which is perhaps involved only through processes like those which determine its distribution between cells and plasma in blood, suggest how far-reaching are the physico-chemical changes accompanying muscular activity. But it is easy to see, even from a consideration of the breadth of the respiratory cycle of the blood alone, that the interactions between all the fluids of the body must be greatly increased in this condition.

During work the passage of carbonic acid and oxygen across the capillary walls is greatly modified. For carbonic acid the pressure gradient is much increased, and, if we assume a close approach to equilibrium, for this substance, between the cells of the tissues and venous blood, as is the case for alveolar air and arterial blood, we must still, when estimating the partial pressure of carbon dioxide in the active muscle, make allowance for all the inactive portions of the body through which blood is also flowing, even though more slowly. The values of pCO_2 for work and rest are as follows:

TABLE 44.

Blood of A.V.B.

	pCO_2	
	A	V
Work	38	54.8
Rest	40	45.4

In view of all the facts, it is hard to believe that the carbon dioxide partial pressure in the active muscles during work is much less than 60 mm. or that, during rest, any large part of the body except possibly the heart, has a partial pressure above 50 mm. The time has not yet come to pursue the questions concerning muscular activity which are thus raised.

Under the influence of a threefold or larger increase in pressure gradient, with the aid of a greatly increased blood flow, there is transported during work of this intensity a quantity of carbonic acid nearly nine times that transported during rest. But the maximum transport attainable by a man of the size of A.V.B. and in the best condition is at least double this amount. Indeed the normal human being experiences little difficulty beyond labored breathing in excreting almost any quantity of carbonic acid which it is possible for him to produce. This is due, as already explained, to the relatively high solubility of gaseous carbon dioxide. For this reason a given head of carbon dioxide pressure leads to diffusion twenty or even thirty-fold greater than does the same head of oxygen pressure. Hence when movement of oxygen is barely sufficient, movement of carbon dioxide takes place with a large margin of safety, and substantial reduction of the pressure gradient, though it would tend to increase the carbonic acid content and the hydrogen ion concentration of the blood, would in other respects little modify the total carbonic acid excretion.

The movement of oxygen is attended by far greater difficulties and, as the work of Hill has shown, it may become very insufficient, thus leading to the condition of oxygen debt which he has described. Even in the state of less intense metabolism with which we are now concerned there are changes which show that the resources for the transport of oxygen are not unlimited. In the lung these changes are less conspicuous, in the tissues more so. In order to fix our ideas we may take the following readings from the nomograms:

TABLE 45.

Blood of A.V.B.

	pO_2	
	A	V
Work	75	31
Rest	78	40

The conspicuous fact is the decrease of one-fourth of partial pressure of oxygen in mixed venous blood, accompanying the change from rest to work. The decrease in partial pressure of oxygen in venous blood *in the active muscle* is, however, probably much less than this and may be negligible, but the information necessary to settle this question is lacking. In default of measurements of the composition of venous blood from different organs, the following hypothetical considerations will perhaps serve a useful purpose. Let the utilization of oxygen in the active and also in the inactive portions of the body be constant, say 4 volumes per cent for inactive portions of the body and 12 volumes per cent for active portions of the body. Then, if during rest about one-tenth of the blood flows through active portions and during work this proportion is increased to about three-fourths, the results would be approximately those described by the nomogram. Utilization of 12 volumes per cent of oxygen would involve a venous oxygen pressure of about 25 mm. This computation is presented merely for purposes of illustration and I wish explicitly to disclaim any implication that it may be used except as a means of describing the trend of the changes. In Chapter XII there may be found, however, a further analysis of this question, based upon the most recent work of Bock and Dill.

On general grounds, it may, however, be assumed that before fatigue sets in conditions within the active muscle are fairly constant and, in particular, that constant oxygen pressure should make for efficiency, in accordance with the theory of the *milieu intérieur*. Our observations on the value of the coefficient of utilization of oxygen as

a function of metabolic rate are also consistent with the hypothesis of a roughly constant oxygen pressure in the venous blood of the active muscle. These observations, which are very similar for all individuals who have been studied, are presented on figure 193 for the case of A.V.B.

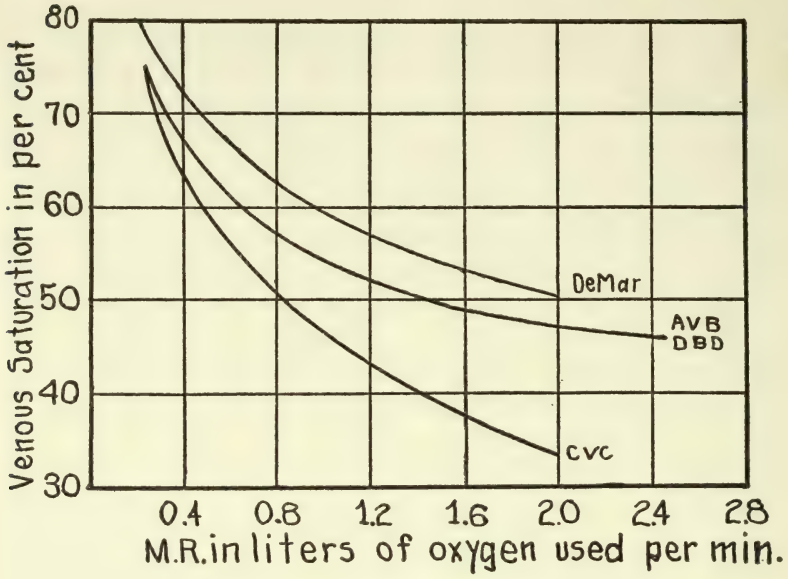

Fig. 193

Venous Oxygen Saturation and Metabolic Rate

Evidently, as the metabolic rate increases, the total coefficient of utilization of oxygen approaches asymptotically a limiting value of about 50 per cent and this limiting value may be regarded as slightly less than that of the venous blood of the active muscle in extreme hard work. Needless to say, such a consideration does not prove that conditions in the muscle are even approximately changeless, but it is consistent with such a view.

In any case, the partial pressure of oxygen in venous blood from the active muscle is probably lower than that

of the mixed venous blood and may be assumed to have the value of about 25 mm. This would involve a value of pCO_2 of about 60 mm. Since in making this calculation certain important factors have been neglected, the head of oxygen pressure at the venous end of a muscle capillary is probably less than 25 mm. and it is evident that a small decrease in venous oxygen pressure must be accompanied by a large decrease in the relative magnitude of the head of pressure. In view of these considerations, and regardless of the validity of the hypothesis that the oxygen pressure of blood in the active muscle has remained relatively constant, it seems to be certain that a considerable fall in the oxygen pressure of mixed venous blood below that of the present case would be incompatible with physiological efficiency, if not impossible.

We may compute the mean head of oxygen pressure, $\Delta p'$, for the lungs and the tissues as a whole in the manner described in Chapter VIII. The result of this computation is given in table 46 for the lungs, assuming the value of 110 mm. for pO_2 in alveolar air, and, for the tissues, assuming two values 0 and 20 mm. for the pressure of oxygen in the interior of the cells.

TABLE 46.

Value of $\Delta p'$.

	LUNG	TISSUE	
		$pO_2 = 0$ mm.	$pO_2 = 20$ mm.
Rest	53	51	30
Work	56	45	23

The values contained in this table are subject to the criticisms which were discussed in treating the problem of diffusion. If it be true that the conditions in the active muscle remain roughly constant, the difference between rest and work shown in the second and third columns of the table are due to differences in the distribution of the blood in rest and work and to a lower value of $\Delta p'$ as thus

calculated for the active muscle than for the inactive parts of the body in general.

In the present chapter we have discussed in detail but a single case of work. In addition, however, the facts concerning the variation in oxygen capacity, in the level of the carbon dioxide dissociation curve, and in the respiratory quotient as functions of metabolic rate for the range of oxygen consumption between 250 cc. and 2500 cc. per minute have been studied. With the help of this information, interpolation, and slight extrapolation will readily yield sufficient information concerning all the properties of blood and of its respiratory cycle for nearly the whole of this range in the case of A.V.B. Other individuals differ sensibly from A.V.B. according to their condition, their size, and other factors, but we possess experimental evidence in abundance to show that the present instance is fairly typical of a healthy man who is leading the ordinary life of the city and is not in training.

CHAPTER X

DISEASE

WE have now to describe the blood of pathological states. Unlike the relatively slight, more or less uniform, and transitory, changes in blood which follow increase of the metabolic rate the changes which occur in disease are often large and persistent and also various. Some of these have been recognized and studied for a long time.

The most conspicuous among pathological variations in the composition of blood is anemia, a condition known to physicians, or at least apparent, in all ages, which received a partial rational interpretation as soon as the function of hemoglobin in the transport of oxygen was discovered. The term anemia is applied to any case in which the concentration of hemoglobin in the blood is considerably diminished below what is regarded as its normal value. This may depend upon a mere reduction in the number of red cells, both cells and plasma remaining approximately normal, or the concentration of hemoglobin in the cells may be reduced, and there may also be other changes in the cells. Anemia may occur alone or accompanied by modifications of the other properties of the blood. In the less familiar disease known as polycythemia the number of red cells and the concentration of hemoglobin in the blood are increased.

The condition known as acidosis is today hardly less familiar than anemia. In acidosis the capacity of the blood to absorb carbonic acid is sensibly diminished. The changes of the blood in this condition were first quantitatively described by pupils of Schmiedeberg[131] and then

[131] F. Walter, *Archiv für experimentelle Pathologie und Pharmakologie,* I, 148 (1877); H. Meyer, *Ibid.,* XVII, 304 (1883); O. Minkowski, *Ibid.,* XIX, 209 (1885).

interpreted from the physico-chemical standpoint by means of the theory of acid-base equilibrium.[132] After the discussion of this topic in the third chapter of the present book, the question would call for little comment were it not that the term acidosis was originally applied to the condition produced by the action of β-hydroxybutyric acid and acetoacetic acid upon the blood in diabetes, and that it is still believed by many that in this condition the

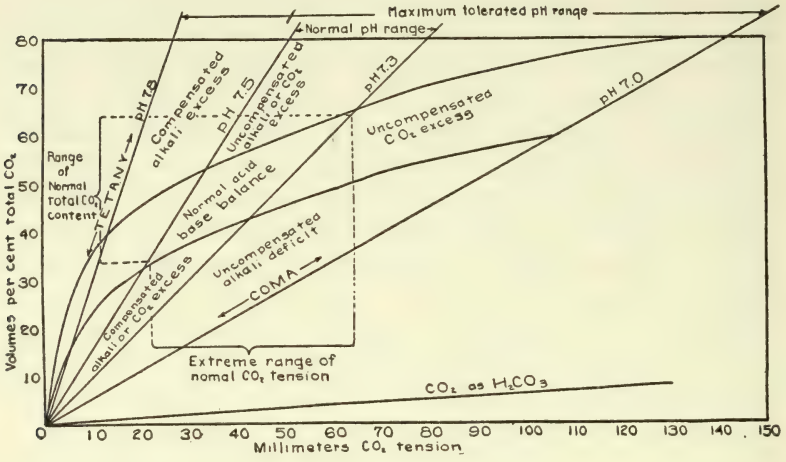

Fig. 194

Classification of Variations of Blood Carbonic Acid

hydrogen ion concentration of the blood is necessarily increased. Reference to Chapter III will show that this is not always the case even when, through the action of acid, the sum $(BP)_B + (BHCO_3)_B$ is reduced. As we have seen, it is this sum which best measures the carbonic acid capacity of the blood when the value of $(P)_B$ is either normal, or constant for the conditions which are being compared.

[132] L. J. Henderson, *Ergebnisse der Physiologie, Jahrgang* VIII, p. 318 (1909).

In order to avoid a long discussion we may take advantage of a chart of Van Slyke's in which the various possible conditions of the circulating blood in respect to these changes are graphically defined. On this figure the condition sometimes called alkalosis, where the carbonic acid capacity of blood is increased, is also described.

Anemia and acidosis are, from the clinical standpoint, the most interesting modifications of the physico-chemical system of blood, in that they involve large variations in the two great functions of oxygen and carbonic acid transport. But under pathological conditions every component of the system is liable to variation. In nephritis the osmotic pressure is often increased, the concentration of serum protein often diminished. Variations in chloride and in base frequently occur, and variations in partial pressure of oxygen and carbon dioxide are very common. None of these changes can take place without leading to other modifications of the system and without changes in its respiratory activity. In view of all these complications, we must once more turn to the nomographic method of description.

The work of my collaborators at the Massachusetts General Hospital has included a comparative study of the blood in a number of different diseases. Among these one has already been employed in explaining the method of constructing a complete nomogram. This is a case of pernicious anemia for which we possess a description of the blood on three occasions: (1) At the time of admission to the hospital when the blood was in a very abnormal condition, the hemoglobin concentration being only about one-third normal; (2) a month later when the hemoglobin concentration had nearly doubled; (3) three weeks later when the blood had almost regained a normal composition.

The results of these studies are presented in figures 195, 196, and 197, and in tables 47 to 57.

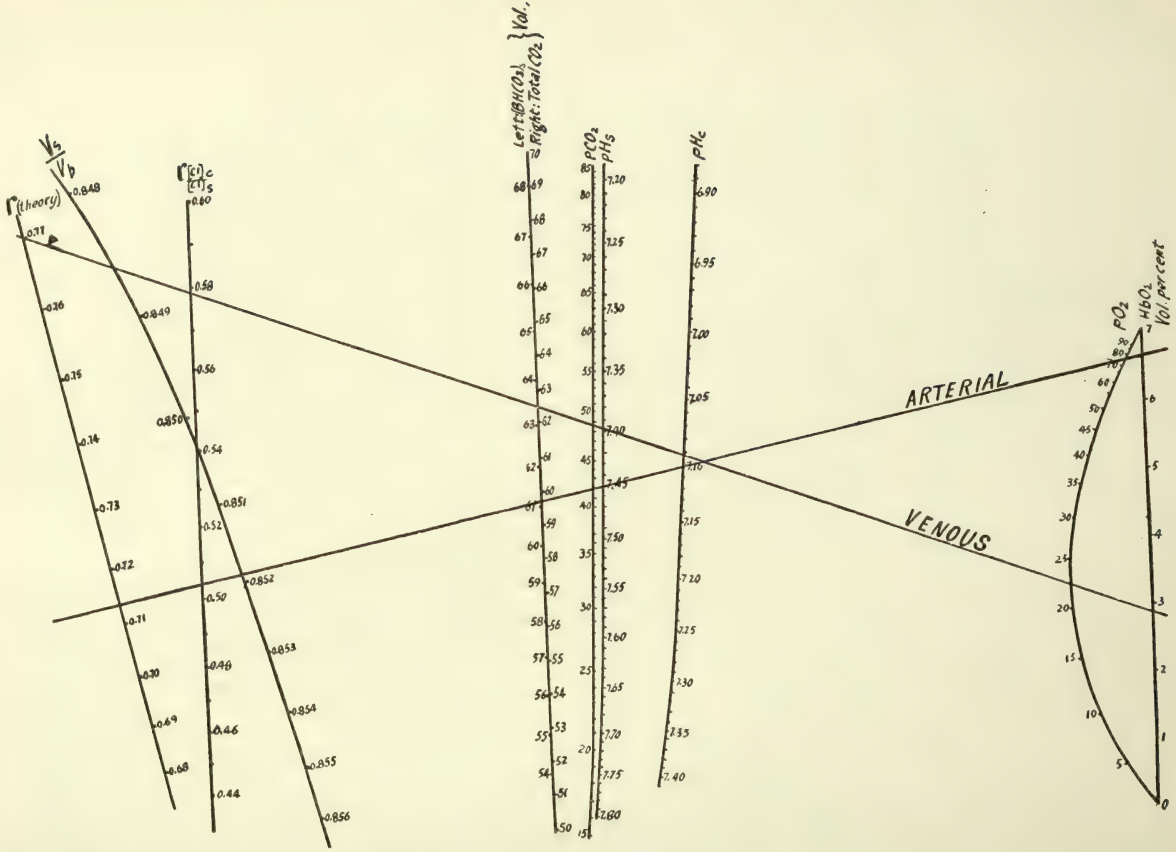

Fig. 195

Blood of Pernicious Anemia: Severe Stage

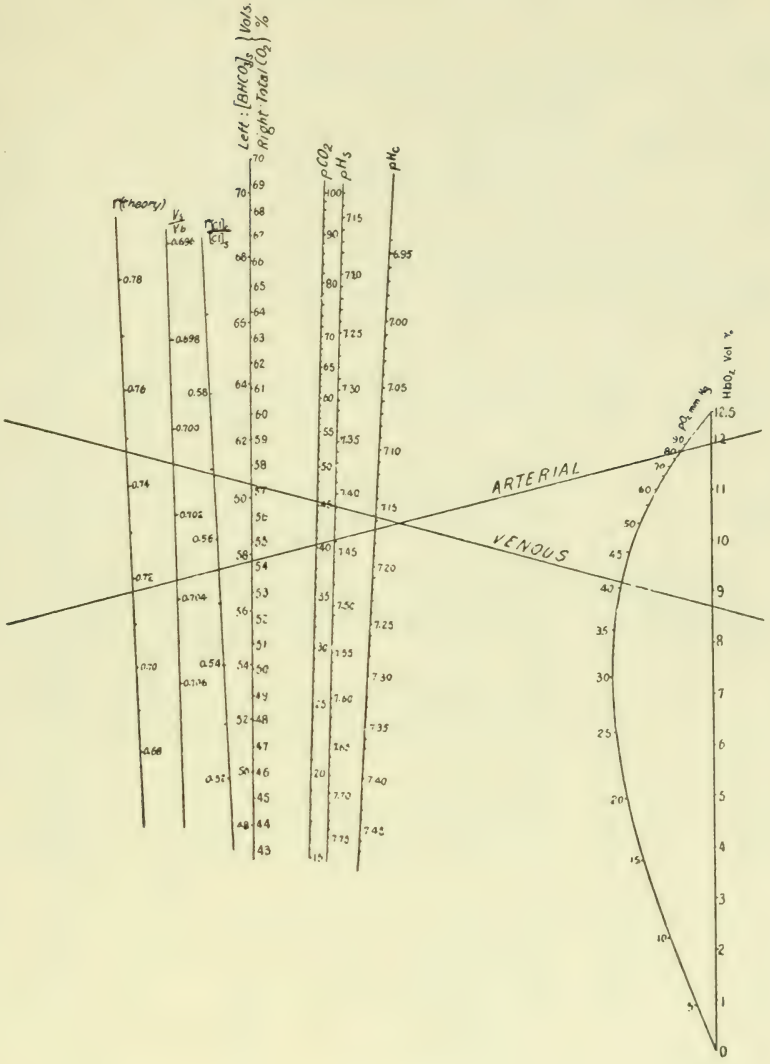

Fig. 196

Blood of Pernicious Anemia: Partial Recovery

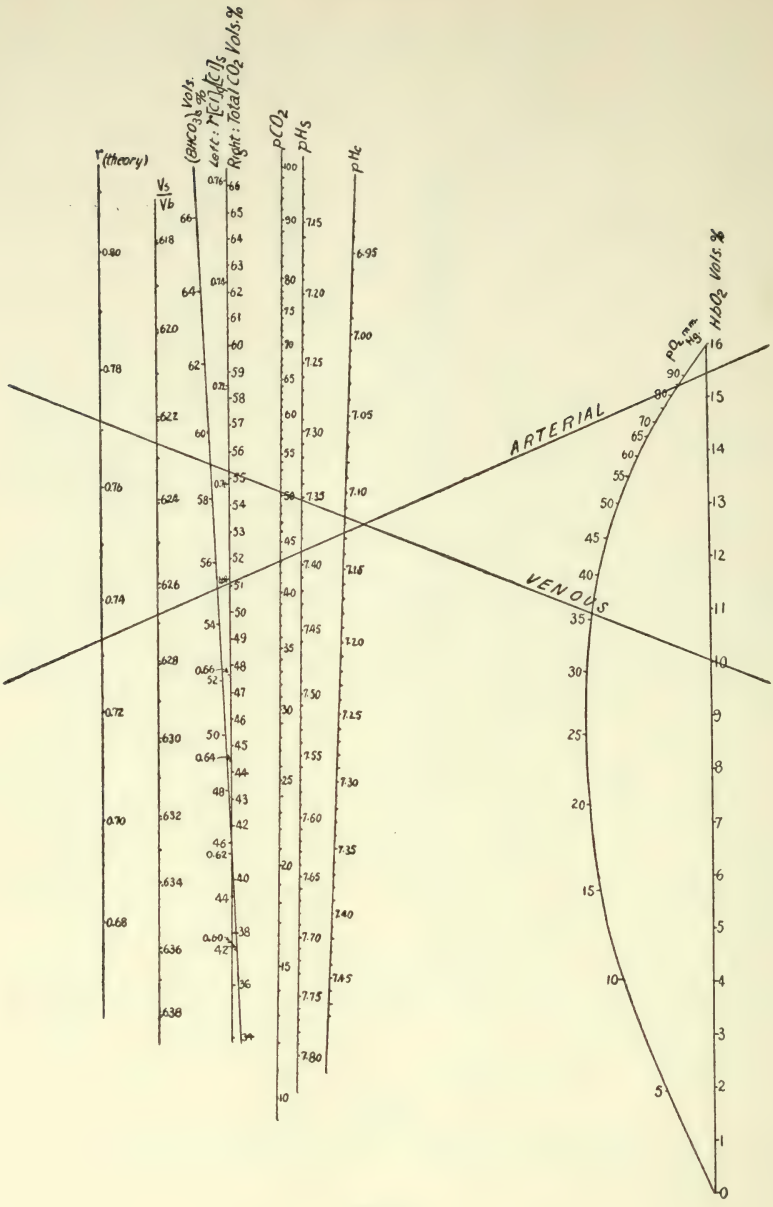

Fig. 197

Blood of Pernicious Anemia: Further Recovery

TABLE 47.

Blood of T.J.F. on January 28, 1927.

Concentration of hemoglobin = 3.13 *mM* per liter of blood.
Concentration of serum proteins = 59.4 gm. per liter of blood.
Respiratory quotient = 0.70.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	800.6	106.4	907	797.4	109.6	907.0	−3.2	+3.2	0.0
B *mM* " " "	124.38	18.75	143.13	124.38	18.75	143.13			
X " " " "	1.81	2.97	4.78	2.24	2.86	5.10	+0.43	−0.11	+0.32
Cl " " " "	88.68	5.92	94.60	87.63	6.97	94.60	−1.05	+1.05	0.0
BP " " " "	10.66	7.66	18.32	10.48	6.48	16.96	−0.18	−1.18	−1.36
$BHCO_3$ " " " "	23.23	2.20	25.43	24.03	2.44	26.47	+0.80	+0.24	+1.04
$BHCO_3$, *vol. per cent*	52.05	4.93	56.98	53.82	5.47	59.29	+1.77	+0.54	+2.31
H_2CO_3, *mM per l. blood*	1.09	0.15	1.24	1.26	0.17	1.43	+0.17	+0.02	+0.19
H_2CO_3, *vol. per cent*	2.44	0.33	2.77	2.82	0.39	3.21	+0.38	+0.06	+0.44
Total CO_2, *mM per l. blood* ..	24.32	2.35	26.67	25.29	2.61	27.90	+0.97	+0.26	+1.23
Total CO_2, *vol. per cent*	54.49	5.26	59.75	56.64	5.86	62.50	+2.15	+0.60	+2.75
Free O_2, *mM per l. blood*			0.09			0.03			−0.06
Free O_2, *vol. per cent*			0.21			0.06			−0.15
Combined O_2, *mM per l. blood*		2.97	2.97		1.28	1.28		−1.69	−1.69
Combined O_2, *vol. per cent* ..		6.65	6.65		2.88	2.88		−3.77	−3.77
Total O_2, *mM per l. blood* ..			3.06			1.31			−1.75
Total O_2, *vol. per cent*			6.86			2.94			−3.92
pCO_2, *mm. Hg*			42.0			48.7			+6.7
pO_2, *mm. Hg*			76.0			22.5			−53.5
Volume, *cc. per l. blood*	851.8	148.2	1,000.0	848.6	151.4	1,000.0	−3.2	+3.2	
pH	7.450	7.100		7.398	7.092		−0.052	−0.008	
r_{theory}			0.713			0.770			+0.057
r_{HCO_3}			0.694			0.738			+0.044
r_{Cl}			0.503			0.579			+0.076

TABLE 48.

Serum of T.J.F. on January 28, 1927.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	939.9	939.8	−0.1
B	*mM* " " "	146.00	146.60	+0.60
X	" " " "	2.13	2.64	+0.51
Cl	" " " "	104.08	103.29	−0.53
BP	" " " "	12.51	12.35	−0.16
$BHCO_3$	" " " "	27.28	28.32	+1.04
H_2CO_3	" " " "	1.28	1.49	+0.21
Total CO_2	" " " "	28.56	29.81	+1.25

TABLE 49.

Cells of T.J.F. on January 28, 1927.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	718.0	723.8	+5.8
B	*mM* " " "	126.54	123.85	−2.69
X	" " " "	20.05	18.89	−1.16
Cl	" " " "	39.94	46.03	+6.09
BP	" " " "	51.70	42.81	−8.89
$BHCO_3$	" " " "	14.85	16.12	+1.27
H_2CO_3	" " " "	1.01	1.12	+0.11
Total CO_2	" " " "	15.86	17.24	+1.38
Combined O_2	" " " "	20.02	8.45	−11.57
Total Hb	" " " "	21.10	20.67	−0.43

TABLE 50.

Blood of T.J.F. on March 1, 1927.

Concentration of hemoglobin = 5.58 *mM* per liter of blood.
Concentration of serum proteins = 47.7 gm. per liter of blood.
Respiratory quotient = 0.92.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	662.0	215.3	877.3	659.3	218.0	877.3	−2.7	+2.7	0.0
B *mM* " " "	104.82	33.48	138.30	104.82	33.48	138.30			
X " " " "	3.71	0.09	3.80	3.51	0.31	3.82	−0.20	+0.22	+0.02
Cl " " " "	74.41	13.43	87.84	73.91	13.93	87.84	−0.50	+0.50	0.0
BP " " " "	8.55	15.03	23.58	8.46	13.90	22.36	−0.09	−1.13	−1.22
$BHCO_3$ " " " "	18.15	4.93	23.08	18.94	5.34	24.28	+0.79	+0.41	+1.20
$BHCO_3$, *vol. per cent*	40.66	11.05	51.71	42.41	11.97	54.38	+1.75	+0.92	+2.67
H_2CO_3, *mM per l. blood*	0.87	0.28	1.15	0.97	0.32	1.29	+0.10	+0.04	+0.14
H_2CO_3, *vol. per cent*	1.94	0.63	2.57	2.18	0.72	2.90	+0.24	+0.09	+0.33
Total CO_2, *mM per l. blood* ..	19.02	5.21	24.23	19.91	5.66	25.57	+0.89	+0.45	+1.34
Total CO_2, *vol. per cent*	42.60	11.68	54.28	44.59	12.69	57.28	+1.99	+1.01	+3.00
Free O_2, *mM per l. blood*			0.09			0.05			−0.04
Free O_2, *vol. per cent*			0.21			0.11			−0.10
Combined O_2, *mM per l. blood*		5.30	5.30		3.88	3.88		−1.42	−1.42
Combined O_2, *vol. per cent* ..		11.87	11.87		8.70	8.70		−3.17	−3.17
Total O_2, *mM per l. blood* ...			5.39			3.93			−1.46
Total O_2, *vol. per cent*			12.08			8.81			−3.27
pCO_2, *mm. Hg*			40.4			45.5			+5.1
pO_2, *mm. Hg*			80.0			41.0			−39.0
Volume, *cc. per l. blood*	703.5	296.5	1,000.0	700.8	299.2	1,000.0	−2.7	+2.7	
pH	7.438	7.167		7.410	7.157		−0.028	−0.010	
r_{theory}			0.717			0.747			+0.030
r_{HCO_3}			0.836			0.854			+0.018
r_{Cl}			0.555			0.570			+0.015

TABLE 51.

Serum of T.J.F. on March 1, 1927.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	941.0	940.9	−0.1
B	*mM* " " "	149.0	149.58	+0.58
X	" " " "	5.27	5.01	−0.26
Cl	" " " "	105.78	105.48	−0.30
BP	" " " "	12.15	12.07	−0.08
$BHCO_3$	" " " "	25.80	27.02	+1.22
H_2CO_3	" " " "	1.24	1.38	+0.14
Total CO_2	" " " "	27.04	28.40	+1.36

TABLE 52.

Cells of T.J.F. on March 1, 1927.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	726.0	728.6	+2.6
B	*mM* " " "	112.94	111.89	−1.15
X	" " " "	0.30	1.04	+0.74
Cl	" " " "	45.30	46.58	+1.28
BP	" " " "	50.72	46.43	−4.29
$BHCO_3$	" " " "	16.62	17.84	+1.22
H_2CO_3	" " " "	0.94	1.07	+0.13
Total CO_2	" " " "	17.56	18.91	+1.35
Combined O_2	" " " "	15.71	11.61	−4.10
Total Hb	" " " "	16.55	16.70	+0.15

TABLE 53.

Blood of T.J.F. on March 23, 1927.

Concentration of hemoglobin = 7.14 *mM* per liter of blood.
Concentration of serum proteins = 42.1 gm. per liter of blood.
Respiratory quotient = 0.76.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	591.2	273.8	865.0	587.0	278.0	865.0	−4.2	+4.2	0.0
B *mM* " " "	95.90	42.80	138.70	95.90	42.80	138.70			
X " " " "	8.23	−2.02	6.21	8.64	−2.15	6.49	+0.41	−0.13	+0.28
Cl " " " "	64.82	20.38	85.20	63.54	21.66	85.20	−1.28	+1.28	0.0
BP " " " "	7.41	18.28	25.69	7.30	16.46	23.76	−0.11	−1.82	−1.93
$BHCO_3$ " " " "	15.44	6.16	21.60	16.42	6.83	23.25	+0.98	+0.67	+1.65
$BHCO_3$, *vol. per cent*	34.59	13.79	48.38	36.78	15.30	52.08	+2.19	+1.51	+3.70
H_2CO_3, *mM per l. blood*	0.83	0.38	1.21	0.96	0.46	1.42	+0.13	+0.08	+0.21
H_2CO_3, *vol. per cent*	1.85	0.85	2.70	2.16	1.02	3.18	+0.31	+0.17	+0.48
Total CO_2, *mM per l. blood* ..	16.27	6.54	22.81	17.38	7.29	24.67	+1.11	+0.75	+1.86
Total CO_2, *vol. per cent*	36.44	14.64	51.08	38.94	16.32	55.26	+2.50	+1.68	+4.18
Free O_2, *mM per l. blood*			0.10			0.04			−0.06
Free O_2, *vol. per cent*			0.23			0.09			−0.14
Combined O_2, *mM per l. blood*		6.90	6.90		4.49	4.49		−2.41	−2.41
Combined O_2, *vol. per cent* ..		15.45	15.45		10.06	10.06		−5.39	−5.39
Total O_2, *mM per l. blood* ...			7.00			4.53			−2.47
Total O_2, *vol. per cent*			15.68			10.15			−5.53
pCO_2, *mm. Hg*			43.1			50.6			+7.1
pO_2, *mm. Hg*			85.0			36.0			−49.0
Volume, *cc. per l. blood*	626.8	373.2	1,000.0	622.6	377.4	1,000.0	−4.2	+4.2	
pH	7.390	7.127		7.352	7.116		−0.038	−0.011	
r_{theory}			0.733			0.772			+0.039
r_{HCO_3}			0.860			0.878			+0.018
r_{Cl}			0.679			0.720			+0.041

TABLE 54.

Serum of T.J.F. on March 23, 1927.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	943.0	942.8	−0.2
B	*mM* " " "	153.0	154.0	+1.0
X	" " " "	13.13	13.88	+0.75
Cl	" " " "	103.42	102.02	−1.40
BP	" " " "	11.82	11.72	−0.10
$BHCO_3$	" " " "	24.63	26.38	+1.75
H_2CO_3	" " " "	1.32	1.54	+0.22
Total CO_2	" " " "	25.95	27.92	+1.97

TABLE 55.

Cells of T.J.F. on March 23, 1927.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	733.8	736.5	+2.7
B	*mM* " " "	114.70	113.40	−1.30
X	" " " "	−5.41	−5.70	−0.29
Cl	" " " "	54.62	57.39	+2.77
BP	" " " "	48.99	43.61	−5.38
$BHCO_3$	" " " "	16.50	18.10	+1.60
H_2CO_3	" " " "	1.02	1.22	+0.20
Total CO_2	" " " "	17.52	19.32	+1.80
Combined O_2	" " " "	18.49	11.90	−6.59
Total Hb	" " " "	19.13	18.92	−0.21

TABLE 56.

Arterial serum of T.J.F. during recovery from pernicious anemia.

		A January 28	B March 1	C March 23	Δ
H_2O	*cc. per l. serum*	939.9	941.0	943.0	−3.1
B	*mM* " " "	146.00	149.00	153.00	−7.0
X	" " " "	2.13	5.27	13.13	−11.00
Cl	" " " "	104.08	105.78	103.42	+0.66
BP	" " " "	12.51	12.15	11.82	+0.69
$BHCO_3$	" " " "	27.28	25.80	24.63	+2.65
H_2CO_3	" " " "	1.28	1.24	1.32	−0.04
Total CO_2	" " " "	28.56	27.04	25.95	+2.61
pH	.....	7.45	7.438	7.39	+0.06
Protein	*gm. per l. serum*	69.6	67.8	66.9	+2.7

TABLE 57.

Arterial cells of T.J.F. during recovery from pernicious anemia.

		A January 28	B March 1	C March 23	Δ
H_2O	*cc. per l. cells* ...	718.0	726.0	733.8	−15.8
B	*mM* " " " ...	126.54	112.94	114.70	+11.84
X	" " " " ...	20.05	0.30	−5.41	+25.46
Cl	" " " " ...	39.94	45.30	54.62	−14.68
BP	" " " " ...	51.70	50.72	48.99	+2.71
$BHCO_3$	" " " " ...	14.85	16.62	16.50	−1.65
H_2CO_3	" " " " ...	1.01	0.94	1.02	−0.01
Total CO_2	" " " " ...	15.86	17.56	17.52	−1.66
Combined O_2	" " " " ...	20.02	17.87	18.49	+1.53
Total Hb	" " " " ...	21.10	18.82	19.13	+1.97
pH		7.10	7.167	7.127	−0.027

As a first rough approximation, the variations from the normal of this case of pernicious anemia may be attributed to the variations in the relative amounts of cells and plasma, or, in other words, the condition may be regarded as nothing but a state of simple anemia. Nevertheless even a superficial examination of the facts will show that to this variation only the general trend and the order of magnitude of the variations can be attributed. Tables 56 and 57 enable us to scrutinize the question more closely. They give the composition of serum and of cells for arterial blood in the three stages of the present case. Thus a quantitative comparison is possible.

It must not be forgotten, however, that really precise comparisons are in some respects impossible when we study cells and plasma separately, for the reason that each phase is in a state of equilibrium with and, therefore, influenced by the other and that as blood varies in composition it is impossible to find strictly corresponding states of equilibrium for comparison. Nevertheless, in these four instances, the conditions of arterial cells and serum are sufficiently close to corresponding states for our present purposes. The difficulty may be illustrated by

a consideration of the values for blood water, cell water, and serum water in the present case. Blood water is high in pernicious anemia because cell volume is low and serum volume high, and because cell water and serum water vary chiefly with cell volume and serum volume. For example, in this case, on January 28 water was 907 cc. per liter blood and cell volume 148 cc. per liter blood, while in the blood of A.V.B. water is 848 cc. per liter blood and cell volume 400 cc. per liter blood. Thus it is apparent that the values of the one variable partially explain those of the other. But, at the same time, in T.J.F. water was 940 cc. per liter serum and 718 cc. per liter cells, in A.V.B. 943 cc. per liter serum and only 705 cc. per liter cells. Hence, if we may regard the two arterial bloods as existing in corresponding states, which is a convenient and justifiable assumption, we see that in pernicious anemia concentration of water in serum is relatively low, in cells high. Reference to the values for March 1 and March 23 shows that the first of these variables approaches the normal while the second moves away from the normal during the recovery period in this case.

But, if we return to conditions on January 28 and consider the variations of the composition of the blood as pO_2 and pCO_2 vary, it is apparent that a state of equilibrium may be found where both cells and serum have more nearly the water content of normal blood. In order to discover this state we have only to make use of the nomogram. Computation shows that, starting with the arterial blood of T.J.F., movement of about 7 cc. of water per liter of blood from cells to plasma would bring about a condition of equilibrium in which water amounts to 704 cc. per liter cells and slightly more than 940 cc. per liter serum. This corresponds to a value of the quantity $\frac{V_s}{V_B}$ of about 0.859 and, even if the blood remains oxygenated, it involves a very low value of pCO_2, say 15 mm., with correspondingly high values of pH_s and pH_c, say about 7.8 and 7.4 re-

spectively. Such conditions certainly do not occur in this individual and we are, therefore, justified in saying that in this case blood water is high while concentration of water in the cells is also high, but concentration of water in serum is perhaps slightly low. This we say because, for any state which may conceivably be chosen for comparison with normal, such is the case.

Turning to other factors, it is to be noted that for T.J.F. the amount of hemoglobin per liter of cells is low and that this quantity decreases during recovery, as the total hemoglobin of the blood increases. This is also true of the mass of protein per liter of serum. Since in the case of the cells these facts are possibly significant, they are presented more fully in table 58.

Columns 6, 7, and 8 of this table give, in grams, water per liter cells, hemoglobin per liter cells, and water plus hemoglobin per liter cells. It is evident that, while the last of these quantities is nearly normal at the beginning of the treatment, it undergoes a marked decline as the condition of the blood changes. This may be due to increase in the amount of nuclear material contained in the red cells during the stage of rapid increase in the hemoglobin content of the blood. Meanwhile, the sum of serum water plus serum protein per liter serum remains nearly constant at a level about 0.6 per cent below normal. It is impossible to say what may be the cause of this difference, which might be due, for example, to changes in the concentration of fat or of other substances of relatively low osmotic activity.

On January 28, both cells and serum are low in base, later serum base returns to normal while cell base (if the assumptions on which the calculation depends are correct) declines still further. Serum chloride is high in all these conditions, while cell chloride is at first low, at last high. But, since the chloride ion passes back and forth between the two phases, it is better to take account of blood chloride which, at first very high, slowly declines, though re-

TABLE 58.

		I	II	III	IV	V	VI	VII	VIII
		H_2O per l. blood	Hb per l. blood	V Cells per l. blood	$\frac{Hb}{H_2O}$	$\frac{Hb}{V}$	$\frac{H_2O}{V}$	$\frac{\text{grams Hb}}{V}$	$\frac{H_2O}{V} + \frac{\text{grams Hb}}{V}$
Fox	1	106.4	3.13	148.2	29.4	21.1	718	352	1070
	2	215.3	5.58	296.5	25.9	18.8	726	314	1040
	3	273.8	7.14	373.2	26.1	19.1	734	319	1053
A.V.B.		282.0	8.93	400.0	31.7	22.3	705	372	1077

maining high. This variation is largely attributable to the abnormal ratio of cell volume to serum volume.

The level of the carbon dioxide dissociation curves is high in this case, but falls progressively. Nevertheless, the sum $(BP)_B + (BHCO_3)_B$ is low. This quantity rises progressively. The fluctuations are chiefly related to changes in hemoglobin and serum protein concentrations.

The quantity $\Sigma = (B) + (X) + (Cl) + (BHCO_3)$, which roughly measures the osmotic effect of electrolytes, undergoes the variations indicated in table 59.

TABLE 59.

		Fox 1	Fox 2	Fox 3	A.V.B.
Serum	Σ	279.5	285.9	294.2	294.9
	H_2O	940	941	943	943
	Σ/H_2O	297	304	312	313
Cells	Σ	201.4	175.2	180.4	211.0
	H_2O	718	726	734	705
	Σ/H_2O	280	241	246	299

On January 28 the electrolyte concentration is low in both serum and cells, the difference between the two phases being normal. Later this concentration returns to normal in the serum, but meanwhile, in the cells, it seems to fall sharply. Even though estimates of this quantity for the cells were of very uncertain accuracy, because indirect, some variation is not unexpected and may perhaps be related to the presence of reticulated red cells or of cells which have recently passed through this stage. It is, in fact, hardly open to question that water and electrolytes are associated with nuclear material in proportions quite different from those existing in the cell solution.

The oxygen dissociation curves of pernicious anemia are displaced to the right. This fact, first noted by Richards and Strauss,[133] is confirmed by a large number of ob-

[133] Richards and Strauss, *Journal of Clinical Investigation,* IV, 105 (1927).

servations of our own. In order to make the facts as clear as possible, it will be necessary to explain the position of the curve as a function of hydrogen ion concentration. To this end, a roughly approximate mathematical treatment of the problem may be employed.

In the absorption of oxygen four substances are assumed to be involved: Hb, HbO_2, Hb^-, and HbO_2^-.

Let $$[HbO_2] + [HbO_2^-] = C, \tag{1}$$

and $$[Hb] + [Hb^-] = 100 - C. \tag{2}$$

We have, approximately,

$$k_M = \frac{[Hb]}{[HbO_2]} \cdot p^n, \tag{3}$$

and $$k_I = \frac{[Hb^-]}{[HbO_2^-]} \cdot p^n. \tag{4}$$

Also $$\frac{[Hb]}{[Hb^-]} = \frac{[H^+]}{k_R} = 10^{8.18} \cdot [H^+], \tag{5}$$

and $$\frac{[HbO_2]}{[HbO_2^-]} = \frac{[H^+]}{k_O} = 10^{6.62} \cdot [H^+]. \tag{6}$$

From equation 5,

$$[Hb] = 10^{8.18} \cdot [H^+] \cdot [Hb^-]. \tag{7}$$

From equation 6,

$$[HbO_2] = 10^{6.62} \cdot [H^+] \cdot [HbO_2^-]. \tag{8}$$

From equations 2 and 7,

$$[Hb^-] + 10^{8.18} \cdot [H^+] \cdot [Hb^-] = 100 - C.$$

From equations 1 and 8

$$[HbO_2] + 10^{6.62} \cdot [H^+] \cdot [HbO_2^-] = C.$$

Whence $$[Hb^-] = \frac{100 - C}{1 + 10^{8.18} \cdot [H^+]}, \tag{9}$$

and $$[HbO_2] = \frac{C}{1 + 10^{6.62} \cdot [H^+]}. \tag{10}$$

From equations 4, 9, 10

$$k_I = \frac{100 - C}{1 + 10^{8.18} \cdot [H^+]} \times \frac{1 + 10^{6.62} \cdot [H^+]}{C} \times p^n, \qquad (11)$$

and

$$\frac{100 - C}{C} \cdot p^n = k_I \frac{1 + 10^{8.18} \cdot [H^+]}{1 + 10^{6.62} \cdot [H^+]}. \qquad (12)$$

Also

$$\frac{k_I}{k_M} = \frac{k_O}{k_R} = \frac{10^{8.18}}{10^{6.62}} = 10^{1.56}. \qquad (13)$$

If $C = 100 - C$, *i.e.*, if the saturation of hemoglobin be one-half the maximum value, we have

$$p^n = \frac{1 + 10^{8.18} \cdot [H^+]}{1 + 10^{6.62} \cdot [H^+]}. \qquad (14)$$

Therefore, in so far as our postulates are valid, the logarithm of the pressure of oxygen corresponding to half saturation of the blood with oxygen should be approximately a linear function of the quantity $\log f[H^+] = \log \frac{1 + 10^{8.18} \cdot [H^+]}{1 + 10^{6.62} \cdot [H^+]}$.

On figure 198 values of $f[H^+] = \frac{1 + 10^{8.18} \cdot [H^+]}{1 + 10^{6.62} \cdot [H^+]}$ as a function of pH are graphically represented. With the help of this figure and the nomographic descriptions of the

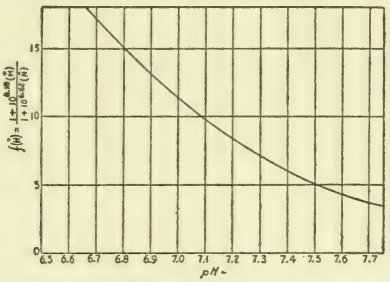

Fig. 198

Theory of Oxygen Effect

blood of a large number of individuals, figure 199 is constructed. On this figure $\log f[H^+]_c = \log \frac{1 + 10^{8.18} \cdot [H^+]_c}{1 + 10^{6.62} \cdot [H^+]_c}$ is plotted against $\log pO_2$, *i.e.*, the logarithm of the pressure of oxygen corresponding to 50 per cent saturation of hemoglobin for the value of hydrogen ion concentration of the corpuscles obtained from the nomograms and used in calculating the values of $\log f[H^+]_c$.

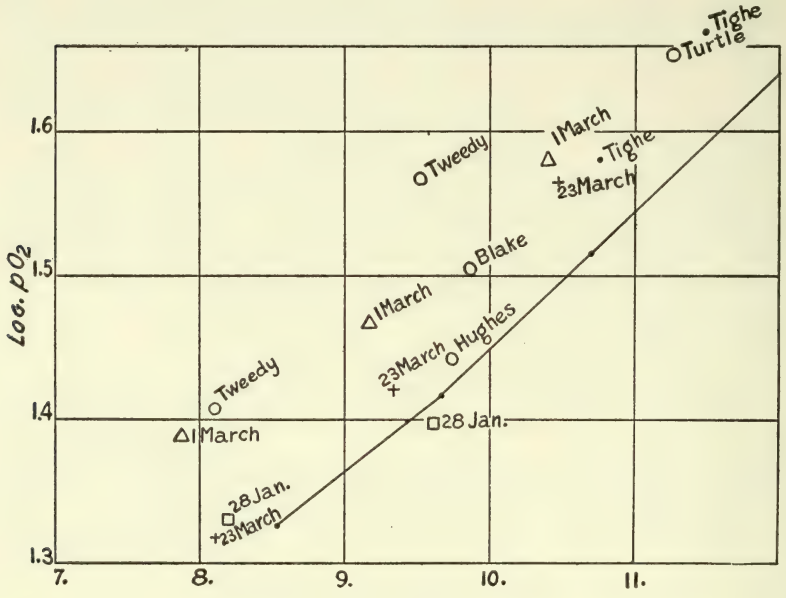

Fig. 199

The Affinity of Hemoglobin for Oxygen

The line upon this figure is drawn through three points giving the average normal values obtained from consistent observations of A.V.B. at rest, A.V.B. at work, and C.V.C. at rest. It will be seen that for normal human blood the relation in question is in fact approximately linear. All other points but one fall to the left of this line. These points include all our studies of pernicious anemia, in-

cluding the blood of T.J.F. on January 28, March 1, and March 23, the blood of T, of H, and of B. There are added observations in the blood of a case of nephritis to be considered later, and on the blood of the snapping turtle.

For all cases of pernicious anemia the average value of log pO_2 (the logarithm of the pressure of oxygen corresponding to half saturation of the blood with oxygen for the calculated value of pH_c) is increased 0.069 above the average value for normal human blood. This amounts to an increase of one-sixth in the oxygen partial pressure and to a corresponding displacement of the oxygen dissociation curves to the right. At present it is impossible to account for these facts. Conceivably, they may be due to variations of the activity coefficients within the cells in this condition. If such variations take place, they must affect not only the apparent values of the constants k_M and k_I of the reaction of hemoglobin with oxygen, but also the apparent ionization constants k'_O and k'_R of the acid radical of hemoglobin. It is hardly to be expected, however, that appreciable effects upon the carbon dioxide dissociation curves will result, since the oxygen effect is approximately at its maximum over a considerable range of hydrogen ion concentration.

We may sum up the result of this discussion in the statement that hemoglobin in the blood of pernicious anemia, of the snapping turtle, and of at least one case of chronic nephritis has a smaller affinity for oxygen than the hemoglobin of normal human blood.

Such a condition makes possible a greater coefficient of utilization of oxygen for given values of pO_2 in arterial and venous bloods and may possibly slightly compensate for diminished oxygen capacity of the blood.

It is this diminished oxygen capacity which is the obvious and perhaps the only very important respiratory disability of the blood in pernicious anemia. On January 28, the blood of T.J.F. carried (*i.e.*, absorbed in the lung and gave off in the tissues) per liter in a single cycle of the circulation only about one-half the amount of oxygen

transported in a normal individual at rest. Accordingly, blood flow must have been almost double the normal blood flow. In spite of this the coefficient of utilization of oxygen was one-third greater than normal and the oxygen pressures of arterial and venous bloods corresponded to those of a normal man during work. Thus the factors of safety of the circulation were reduced on every hand.

Meanwhile the carbonic acid cycle was relatively little modified. Accompanying the increase of bicarbonate, there was a small increase of carbon dioxide partial pressure, with the result that the serum was somewhat alkaline. The cells, however, were slightly more acid than in a normal man. To this disparity between cells and plasma a low value of r corresponds. The range of carbon dioxide pressure was little diminished.

A further discussion of the facts presented in the nomogram and tables is unnecessary and uneconomical. The principal variations have been pointed out and others may be readily found in the tables. But some of these are open to question because of inevitable magnification of small experimental errors in the calculations and, so far as it is now possible to form an opinion, they seem to be relatively unimportant.

In pernicious anemia, the changes of the physico-chemical system are on the whole such as may be attributed to a single cause, reduction in the mass of the cell phase. At least this is true of the larger variations, while the lesser variations are attributable to relatively small changes, especially during the period of recovery, in the composition of this phase. In other pathological states the changes of the system may be more various and not less great. Chronic nephritis, especially, may lead to changes in the blood and in its respiratory function which are almost unparalleled in magnitude and quite unparalleled in diversity. We have studied, during the past year, two cases of this disease at the point of death.[134] In one of these cases,

[134] Henderson, Bock, Dill, Hurxthal, and van Caulaert, *Journal of Biological Chemistry,* LXXV, 305 (1927).

we were able to get a considerable quantity of blood from the heart 15 minutes after death, and, by piecing together the data from all our experiments, it has been possible to construct a complete nomogram. These two cases, although their clinical histories were different, seemed to be almost identical from a pathological standpoint at the time when our studies were made, and, in particular, there was no significant difference in the composition of the bloods. The results of these studies are presented in figure 200 and in tables 60 to 64.

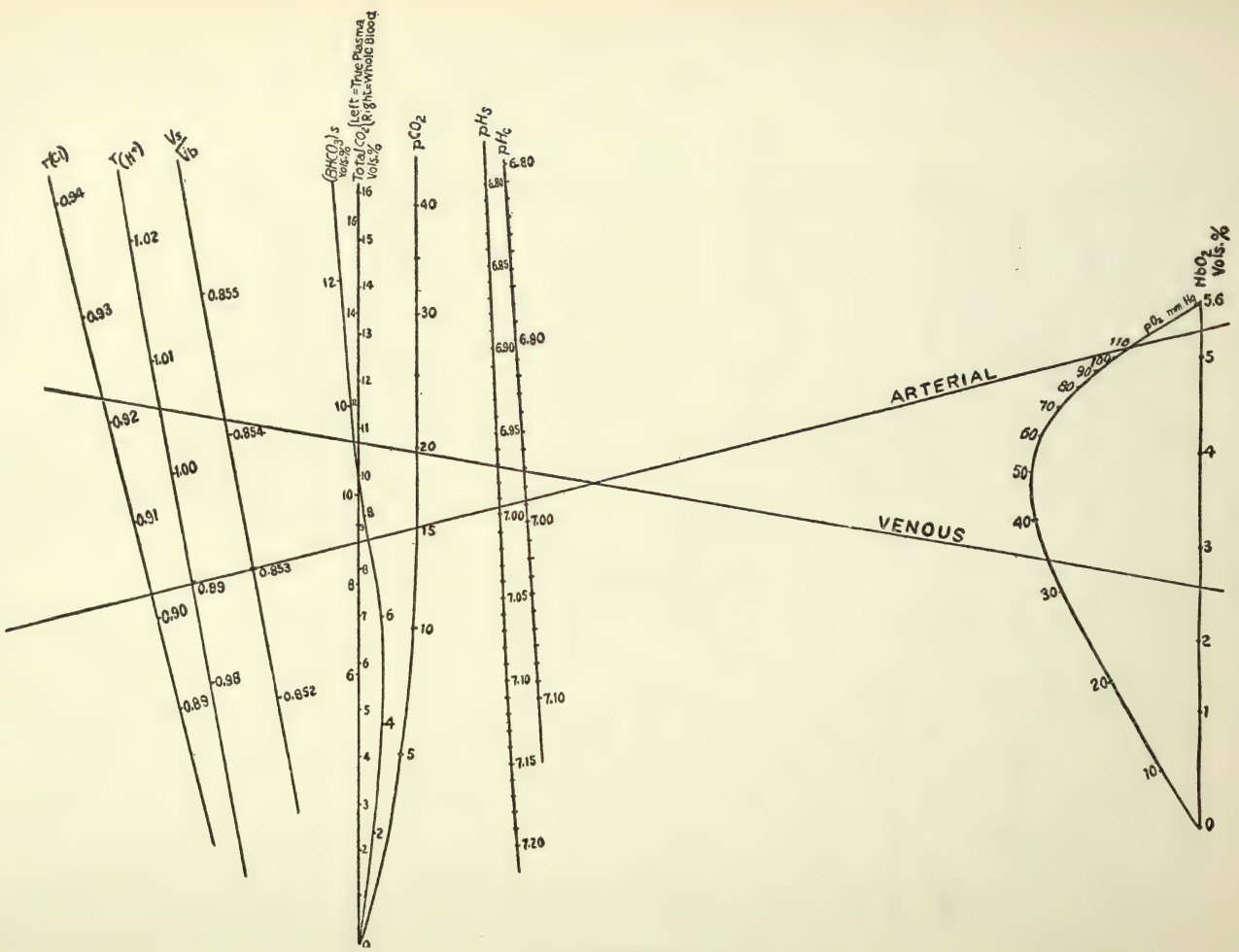

Fig. 200

Blood in Chronic Nephritis

TABLE 60.

Blood of a nephritic at death.

Concentration of hemoglobin = 2.50 *mM* per liter of blood.
Concentration of serum proteins = 44.6 gm. per liter of blood.
Respiratory quotient = 0.73.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	812.0	110.5	922.5	810.9	111.6	922.5	−1.1	+1.1	0.0
B *mM* " " "	134.0	15.0	149.0	134.0	15.0	149.0			
X " " " "	46.35	1.55	47.90	45.80	2.18	47.98	−0.55	+0.63	+0.08
Cl " " " "	78.37	9.63	88.00	78.30	9.70	88.00	−0.07	+0.07	0.0
BP " " " "	6.40	3.30	9.70	6.33	2.49	8.82	−0.07	−0.81	−0.88
$BHCO_3$ " " " "	2.88	0.52	3.40	3.57	0.63	4.20	+0.69	+0.11	+0.80
$BHCO_3$, *vol. per cent*	6.44	1.17	7.61	8.00	1.41	9.41	+1.56	+0.24	+1.80
H_2CO_3, *mM per l. blood*	0.39	0.05	0.45	0.52	0.07	0.59	+0.13	+0.02	+0.15
H_2CO_3, *vol. per cent*	0.88	0.12	1.00	1.16	0.16	1.32	+0.28	+0.04	+0.32
Total CO_2, *mM per l. blood*	3.27	0.58	3.85	4.09	0.70	4.79	+0.82	+0.12	+0.94
Total CO_2, *vol. per cent*	7.32	1.29	8.61	9.16	1.57	10.73	+1.84	+0.28	+2.12
Free O_2, *mM per l. blood*			0.12			0.04			−0.08
Free O_2, *vol. per cent*			0.28			0.09			−0.19
Combined O_2, *mM per l. blood*		2.37	2.37		1.16	1.16		−1.21	−1.21
Combined O_2, *vol. per cent*		5.32	5.32		2.60	2.60		−2.72	−2.72
Total O_2, *mM per l. blood*			2.49			1.20			−1.29
Total O_2, *vol. per cent*			5.60			2.69			−2.91
pCO_2, *mm. Hg*			15.0			19.7			+4.7
pO_2, *mm. Hg*			110.0			35.0			−75.0
Volume, *cc. per l. blood*	853.0	147.0	1,000.0	851.9	148.1	1,000.0	−1.1	+1.1	
pH	6.994	6.987		6.969	6.970		−0.025	−0.017	
r_{HCO_3}			1.33			1.28			−0.05
r_{Cl}			0.903			0.900			−0.003

TABLE 61.

Serum of a nephritic at death.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	952.2	952.0	−0.2
B	*mM* " " "	157.1	157.4	+0.3
X	" " " "	54.4	53.8	−0.6
Cl	" " " "	91.8	92.0	+0.2
BP	" " " "	7.5	7.4	−0.1
$BHCO_3$	" " " "	3.4	4.2	+0.8
H_2CO_3	" " " "	0.5	0.6	+0.1
Total CO_2	" " " "	3.9	4.8	+0.9

TABLE 62.

Cells of a nephritic at death.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	751.7	753.3	+1.6
B	*mM* " " "	102.0	101.3	−0.7
X	" " " "	10.5	14.7	+4.2
Cl	" " " "	65.5	65.5	0.0
BP	" " " "	22.5	16.8	−5.7
$BHCO_3$	" " " "	3.5	4.2	+0.7
H_2CO_3	" " " "	0.3	0.5	+0.2
Total CO_2	" " " "	3.8	4.7	+0.9
Combined O_2	" " " "	16.1	7.8	−8.3
Total Hb	" " " "	17.01	16.89	−0.12

TABLE 63.

Arterial serum of A.V.B. at rest and of a nephritic at death.

		A.V.B.	Nephritic	Δ
H_2O	*cc. per l. serum*	943.3	952.2	+8.9
B	*mM* " " "	154.00	157.10	+3.10
X	" " " "	17.00	54.40	+37.40
Cl	" " " "	99.32	91.80	−7.52
BP	" " " "	13.13	7.50	−5.63
$BHCO_3$	" " " "	24.55	3.40	−21.15
H_2CO_3	" " " "	1.22	0.50	−0.72
Total CO_2	" " " "	25.77	3.90	−21.87
pH		7.425	6.994	−0.431
Protein	*gm. per l. serum*	72.5	54.9	−17.6

TABLE 64.

Arterial cells of A.V.B. at rest and of a nephritic at death.

		A.V.B.	Nephritic	Δ
H_2O	*cc. per l. cells*	705.0	751.7	+46.7
B	*mM* " " "	133.75	102.00	−31.75
X	" " " "	17.73	10.50	−7.23
Cl	" " " "	45.27	65.50	+20.23
BP	" " " "	56.50	22.50	−34.00
$BHCO_3$	" " " "	14.25	3.50	−10.75
H_2CO_3	" " " "	0.93	0.30	−0.63
Total CO_2	" " " "	15.18	3.80	−11.38
Combined O_2	" " " "	21.43	16.10	−5.33
Total Hb	" " " "	22.33	17.01	−5.32
pH		7.124	6.987	−0.137

It is a bewildering task to analyze the pathological changes of this blood, which is physiologically defective in every respect—as an environment of the tissues, as a vehicle for the transport of oxygen, and as a vehicle for the transport of carbonic acid—and which is in hardly any respect unmodified in composition. We may first note that the volume of the cell phase is but little more than one-third of the normal volume and just that of the blood of T.J.F. on January 28 when the anemia was at its worst. But, in the nephritic, the anemia is even more severe, for hemoglobin makes up but 283 grams per liter of cells while, in the case of pernicious anemia, it amounts to 352 grams per liter of cells and, in the normal case of A.V.B., to 369 grams per liter of cells. Thus, the oxygen capacity of the blood is reduced to less than one-third the normal value. The sum of $[BP]_B + [BHCO_3]_B$ is little more than one-fourth the normal value, and, from the chemical standpoint, this is a fair measure of the reduction of the carbonic acid capacity of the blood. But, on account of the reduced partial pressure of carbon dioxide which is but one-third of the normal pressure, as a result of acidosis and greatly increased ventilation of the lung, the carbonic acid content of the blood is hardly more than one-sixth the normal content.

The hydrogen ion concentrations are greatly increased, in the serum nearly threefold, in the cells, however, only about 40 per cent on account of the profound disturbance of the heterogeneous equilibrium between cells and plasma, which finds expression in the increased value of the Donnan r for all ions. The undetermined acids, HX, are also increased nearly threefold, or perhaps more than this, since there is reason to suspect that B_c and X_c are greater than we have estimated them to be. Blood chloride is also slightly increased.

For serum, the value of $\Sigma = (B) + (X) + (Cl) + (BHCO_3)_B$ per liter of water is little, say three per cent, more than normal. But non-protein nitrogen amounts to 4.7 grams per liter blood and this can hardly account for less than an increase in total concentration of the blood solution of 120 millimoles per liter. The osmotic pressure is accordingly one-third greater than normal.

These statements will suffice to show that all the great functions of the blood are deranged and, therefore, to suggest how far-reaching must be the modifications of every part of the body. But they by no means exhaust the analysis of the pathological modifications of the blood.

The serum, like the cells, is low, only about three-fourths normal, in protein content and high in water, but not quite correspondingly high. In spite of the increase in chloride per liter blood, there is a considerable reduction of nine millimoles in the concentration of chloride per liter serum water. Since the value of $(BP) + (BHCO_3)$ per liter serum water is but 11.5 millimoles, it is evident that without this diminution in chloride concentration of serum the transport of carbonic acid could not have continued. The concentration of base per liter serum water, alone among the concentrations of the important components of the system, is normal. But an abnormally large part of this base is associated with X and in view of the insufficient renal action it may be constituted of sodium, potassium, and other ions in abnormal proportions.

We lack direct information concerning the value of B_c, which has been estimated in the conventional manner in accordance with measurements of normal blood. Here such estimates are probably worthless and there is reason to think that a considerable quantity, say 30 millimoles, should be added to the estimates of B and of X per liter cells. The chloride content of the cells is high, measured per liter water about one-third greater than normal. The quantity of base bound by cell protein is enormously reduced, first on account of the great decrease of hemoglobin content, and, secondly, because of the decreased alkalinity of the cells. In spite of the fact that this change of alkalinity is relatively small, while pH_s is much changed, there is a large reduction of base bound per gram of hemoglobin and a small decrease in base bound per gram of serum protein. The facts are as follows:

TABLE 65.

Base bound per gram of protein in arterial blood.

	Serum	Cells
	mM	*mM*
A.V.B.	0.181	0.153
Nephritic	0.143	0.079
Difference	0.038	0.079

They may be readily understood by taking the isoelectric points of the proteins into account. For the serum proteins these points are remote from the hydrogen ion concentration of the serum, while the isoelectric point of hemoglobin is near the reaction of the cell contents. At the isoelectric point a protein is not at all or little dissociated, and the degree of its ionization is approximately a linear function of the quantity pH. Therefore, a small change in pH_c is accompanied by a *relatively* large change in base bound by hemoglobin, while a large change in pH_s is accompanied by a *relatively* small change in base bound by serum protein. The absolute magnitude of the change is

of course dependent upon the buffer value of the protein. For hemoglobin this is very high.

Changes of the respiratory cycle in this blood are also remarkable. The movement of water between cells and plasma, like the accompanying changes in volume of the two phases, is but 1 cc. per liter, one-fourth of the change in the blood of A.V.B. in the resting state. The movement of chloride is merely 0.07 millimoles per liter blood, one-tenth the normal value. The change in total carbonic acid between arterial and venous blood is 0.94 millimoles per liter, the corresponding difference for oxygen 1.29 millimoles per liter. These values may be compared with those for A.V.B. at rest, 1.68 millimoles and 2.05 millimoles respectively; for A.V.B. at work, 4.47 millimoles and 4.47 millimoles respectively; and for T.J.F. on January 28, 0.75 millimoles and 1.07 millimoles respectively. It will be seen that the oxygen carried per liter of blood in the nephritic is about 60 per cent of the amount carried in normal man at rest, 30 per cent of that carried in normal man at work, but 120 per cent of that carried in the blood of T.J.F. The fluctuations in carbonic acid transport depend also upon variation of the respiratory quotient. In the case of nephritis here described the respiratory quotient was assumed to be low.

Variations in the quantity of oxygen carried per liter of blood are significant, especially when considered in relation to oxygen capacity and coefficient of utilization. Relative magnitudes for the four cases in question are in round numbers as follows:

TABLE 66.

	I	II	III	IV
	Oxygen carried per l. blood	Oxygen capacity	Coefficient of utilization of oxygen (I ÷ II)	Venous pO_2
A.V.B. Rest	1.0	1.0	1.0	1.0
A.V.B. Work	2.2	1.1	2.0	0.78
Pernicious Anemia	0.5	0.35	1.4	0.80
Nephritis	0.6	0.28	2.1	0.88

The nephritic, at the point of death, with an organism which is gravely deranged in every respect, transports more oxygen per liter of blood than does the sufferer from pernicious anemia. But, on account of the very low oxygen capacity, this is possible only through an increase of the coefficient of utilization of oxygen to a point which is even higher than that characteristic of A.V.B. in work and which is one-half greater than that observed in pernicious anemia. This condition in the nephritic may perhaps be attributed to a weakened heart, for the coefficient of utilization of oxygen during rest in bed has attained nearly its maximum value and it may even be suspected that a condition of mild anoxemia prevails in certain parts of the body. This is, however, improbable or at least open to question, since increase in the acidity of the corpuscles has decreased the affinity of hemoglobin for oxygen and, therefore, raised the oxygen pressure, especially in venous blood. Moreover, as in pernicious anemia, so in this case of nephritis, there appears to be a further decrease in the affinity of hemoglobin for oxygen for which it is impossible to give an explanation. As a result of the two changes, mixed venous oxygen pressure in the nephritic is 88 per cent of the normal resting value, while it is but 80 per cent of that value in pernicious anemia, with a lower coefficient of utilization of oxygen, and but 78 per cent in the normal man during work. But how the local venous oxygen pressure may vary can hardly be guessed.

Because of the increased breathing, arterial oxygen pressure in the nephritic is very high. This, however, is probably a negligible factor in the present discussion, for, in accordance with the flatness of the upper reaches of the oxygen dissociation curve, this value falls sharply as soon as diffusion of oxygen out of the blood begins. In the nephritic the difference in the value of pH_s for arterial and venous bloods is just equal to that for A.V.B. at rest. This is due to the cancelling out of the effects of many variations, for example, the decrease in concentration of protein tends to increase the variation in pH_s, the in-

crease in coefficient of utilization of oxygen tends to diminish it, the increase of the ratio of the difference of arterial and venous carbon dioxide pressures to the mean value of this pressure tends to increase the difference, and the fall in the respiratory quotient to a value but little greater than 0.78 to diminish it.

The values of pH_c are uncertain. But in them, as in estimates of the concentration of chloride, of X, and of bicarbonate for cells and plasma in arterial and in venous bloods, there is evidence of an unexampled modification of the heterogeneous equilibrium between the two phases of the blood. In round numbers the mean value of the Donnan r for all ions seems to be not far from 1.0, a very large increase over the normal. For bicarbonate the values of r seem to be about 1.3.

The insufficiency of renal action is, no doubt, directly responsible for the twenty-fold increase in non-protein nitrogen, with the resulting great disturbance of osmotic relations. The same cause must be at least partly responsible for the threefold increase in the concentration of X and for the decrease of one-fourth in serum protein concentration. It is safe to assume that the acidosis is also partly a direct result of the renal lesion. But, on the whole, it is manifestly impossible to regard the changes in the blood as direct effects of the pathological state of the kidney. On the contrary, nothing could be clearer than that a disturbance of the whole organism exists. This involves adjustments and readjustments between all the parts and at this stage the breaking point has been reached. There can be little doubt, for instance, that the blood must exert harmful action on the kidney which is hardly less than that exerted by the kidney on the blood. A similar statement will hold good for the heart and the other organs.

In this case we have before us an organism at the moment of dissolution and a complete description of the state of one of its component mechanisms. In spite of the doubtful accuracy of some of the elements of this descrip-

tion, which results from insufficiency of material for analysis, we can readily see that, at best, the state of the blood only barely affords the necessary conditions for the existence of the organism. Yet adaptive properties of some of the pathological changes of the blood may still be recognized. Thus the diminution of chloride content of serum was indispensable to the continuance of carbonic acid excretion. Again the acid reaction of the blood tends to increase oxygen pressure in the tissues and the increased ventilation which it entails raises oxygen pressure in the lung far enough to offset the diminished affinity of hemoglobin for oxygen in the arterial blood. Such changes as these, and perhaps others, all trace of which has disappeared, have prolonged life. It will be possible to conceive more clearly the state of this organism when we take up in Chapter XII the study of diffusion, blood flow, and breathing; for the present we have carried the analysis as far as it may be profitably pursued.

It is also unprofitable to continue the verbal analysis of other pathological states. All that has been said in this chapter and the last concerning the blood in pernicious anemia, in nephritis, and in the normal man during work is either explicitly presented in the nomograms and tables or else easily deducible from them. The verbal analysis is here carried through chiefly to illustrate the meaning and use of these nomograms and tables and it may be hoped that the illustration is now sufficient.

It has been said that mathematics is a language. No doubt the mathematical language of classical physical science is more elegant and more concise than the nomographic language which is employed in this book. But we should reject one of the most precious advantages of mathematics, which is not lacking even in the present exposition, if we were to endeavor to translate into English that which can be well said only in the nomographic idiom. Therefore, the results of a few other pathological and clinical studies are presented in the following pages exclusively in the form of nomograms and tables.

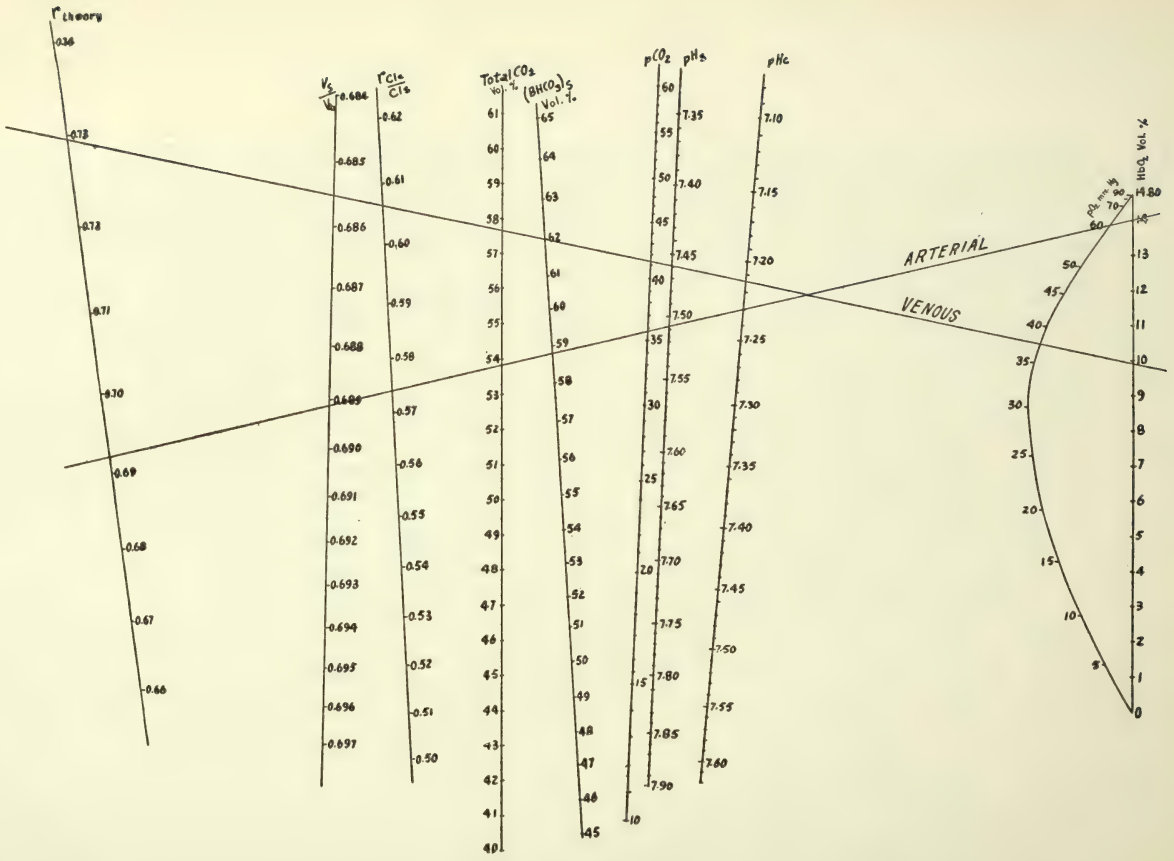

Fig. 201

Blood in Myxedema

In figure 201 the composition of the blood of a typical case of myxedema is represented. For this case the so-called basal metabolic rate was approximately −40 per cent. Tables 67 to 71 complete the description and give a comparison with the condition of the blood in a normal man.

TABLE 67.

Blood in myxedema.

Concentration of hemoglobin = 6.60 *mM* per liter of blood.
Concentration of serum proteins = 52.6 gm. per liter of blood.
Respiratory quotient = 0.91.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	642.1	214.9	857.0	638.5	218.5	857.0	−3.6	+3.6	0.0
B *mM* " " "	103.4	39.6	143.0	103.4	39.6	143.0			
X " " " "	6.63	6.75	13.38	6.75	6.63	13.38	+0.12	−0.12	0.0
Cl " " " "	68.99	13.26	82.25	68.14	14.11	82.25	−0.85	+0.85	0.0
BP " " " "	9.68	14.63	24.31	9.52	13.24	22.76	−0.16	−1.39	−1.55
$BHCO_3$ " " " "	18.10	4.96	23.06	18.99	5.62	24.61	+0.89	+0.66	+1.55
$BHCO_3$, *vol. per cent*	40.55	11.10	51.65	42.54	12.59	55.13	+1.99	+1.49	+3.48
H_2CO_3, *mM per l. blood*	0.75	0.25	1.00	0.86	0.29	1.15	+0.11	+0.04	+0.15
H_2CO_3, *vol. per cent*	1.67	0.56	2.23	1.92	0.65	2.57	+0.25	+0.09	+0.34
Total CO_2, *mM per l. blood* ..	18.85	5.21	24.06	19.85	5.91	25.76	+1.00	+0.70	+1.70
Total CO_2, *vol. per cent*	42.22	11.66	53.88	44.46	13.24	57.70	+2.24	+1.58	+3.82
Free O_2, *mM per l. blood*			0.08			0.05			−0.03
Free O_2, *vol. per cent*			0.19			0.11			−0.08
Combined O_2, *mM per l. blood*		6.27	6.27		4.46	4.46		−1.81	−1.81
Combined O_2, *vol. per cent* ..		14.10	14.10		10.00	10.00		−4.10	−4.10
Total O_2, *mM per l. blood* ...			6.35			4.51			−1.84
Total O_2, *vol. per cent*			14.29			10.11			−4.18
pCO_2, *mm. Hg*			35.9			41.4			+5.5
pO_2, *mm. Hg*			62.0			38.0			−24.0
Volume, *cc. per l. blood*	689.1	310.9	1,000.0	685.5	314.5	1,000.0	−3.6	+3.6	
pH	7.506	7.228		7.458	7.214		−0.048	−0.014	
r_{theory}			0.692			0.730			+0.038
r_{HCO_3}			0.819			0.864			+0.045
r_{Cl}			0.574			0.607			+0.033

TABLE 68.

Serum in myxedema.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	931.9	931.4	−0.5
B	*mM* " " "	150.0	150.84	+0.84
X	" " " "	9.62	9.85	+0.23
Cl	" " " "	100.05	99.40	−0.65
BP	" " " "	14.05	13.89	−0.16
$BHCO_3$	" " " "	26.28	27.70	+1.42
H_2CO_3	" " " "	1.08	1.26	+0.18
Total CO_2	" " " "	27.36	28.96	+1.60

TABLE 69.

Cells in myxedema.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	691.2	694.8	+3.6
B	*mM* " " "	127.33	125.92	−1.41
X	" " " "	21.70	21.08	−0.62
Cl	" " " "	42.64	44.87	+2.23
BP	" " " "	47.04	42.10	−4.94
$BHCO_3$	" " " "	15.95	17.87	+1.92
H_2CO_3	" " " "	0.80	0.92	+0.12
Total CO_2	" " " "	16.75	18.79	+2.04
Combined O_2	" " " "	20.16	14.18	−5.98
Total Hb	" " " "	21.25	20.98	−0.27

TABLE 70.

Arterial serum in normal man and in myxedema.

		Normal man	Myxedema	Δ
H_2O	*cc. per l. serum*	943.3	931.9	−11.4
B	*mM* " " "	154.0	150.0	−4.0
X	" " " "	17.0	9.62	−7.38
Cl	" " " "	99.32	100.05	+0.73
BP	" " " "	13.13	14.05	+0.92
$BHCO_3$	" " " "	24.55	26.28	+1.73
H_2CO_3	" " " "	1.22	1.08	−0.14
Total CO_2	" " " "	25.77	27.36	+1.59
pH		7.425	7.506	−0.081

TABLE 71.

Arterial cells in normal man and in myxedema.

		Normal man	Myxedema	Δ
H_2O	*cc. per l. cells*	705.0	691.2	−13.8
B	*mM* " " "	133.75	127.33	−6.42
X	" " " "	17.73	21.70	+3.97
Cl	" " " "	45.27	42.64	−2.63
BP	" " " "	56.50	47.04	−9.46
$BHCO_3$	" " " "	14.25	15.95	+1.70
H_2CO_3	" " " "	0.93	0.80	−0.13
Total CO_2	" " " "	15.18	16.75	+1.57
Combined O_2	" " " "	21.43	20.16	−1.27
Total Hb	" " " "	22.33	21.25	−1.08
pH		7.124	7.228	+0.104

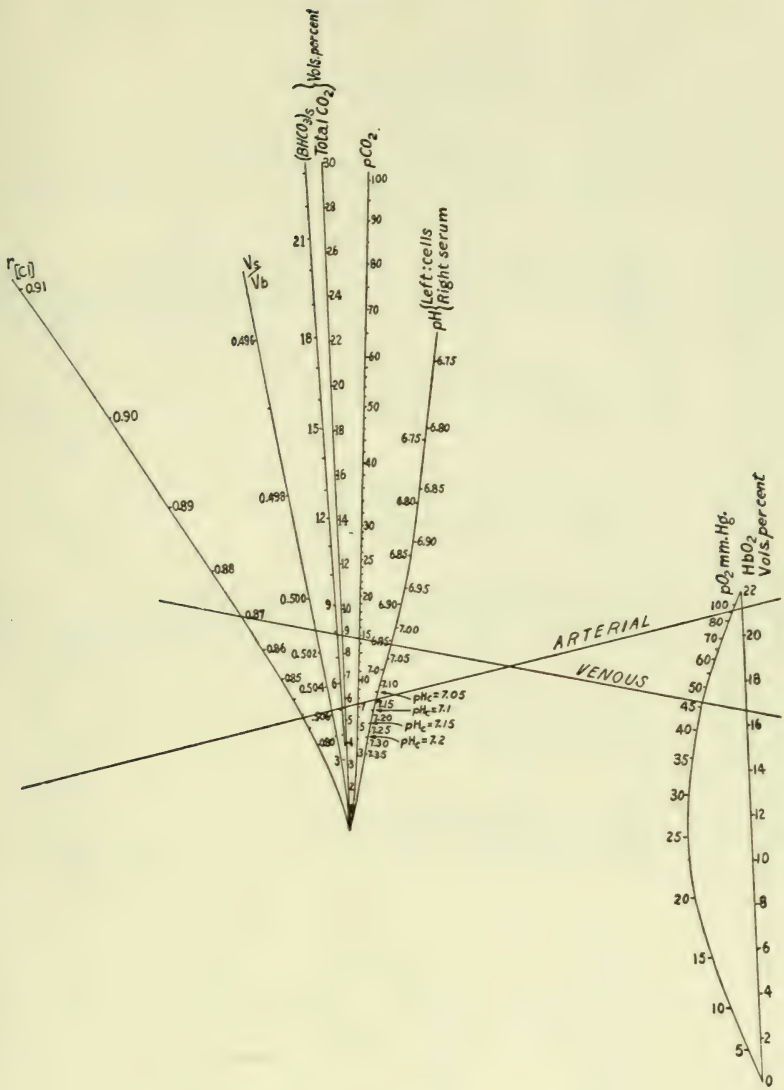

Fig. 202

Blood in Diabetic coma

TABLE 72.

Diabetic coma.

Concentration of hemoglobin = 9.82 *mM* per liter of blood.
Concentration of serum proteins = 32.7 gm. per liter of blood.
Respiratory quotient = 0.69.

	ARTERIAL			VENOUS			Δ		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	467.7	351.3	819.0	463.0	356.0	819.0	−4.7	+4.7	0.0
B *mM* " " "	76.00	58.92	134.92	76.0	58.92	134.92			
X " " " "	15.80	9.50	25.30	16.96	8.35	25.30	+1.15	−1.15	0.0
Cl " " " "	53.83	33.45	87.28	52.32	34.96	87.28	−1.51	+1.51	0.0
BP " " " "	5.20	23.32	28.52	4.95	20.37	25.32	−0.25	−2.97	−3.22
$BHCO_3$ " " " "	1.16	1.15	2.31	1.77	1.79	3.56	+0.61	+0.64	+1.25
$BHCO_3$, *vol. per cent*	2.59	2.58	5.17	3.97	4.00	7.97	+1.38	+1.42	+2.80
H_2CO_3, *mM per l. blood*	0.11	0.08	0.19	0.22	0.17	0.38	+0.11	+0.09	+0.20
H_2CO_3, *vol. per cent*	0.25	0.18	0.43	0.49	0.38	0.87	+0.24	+0.20	+0.44
Total CO_2, *mM per l. blood* ..	1.27	1.23	2.50	1.99	1.96	3.95	+0.72	+0.73	+1.45
Total CO_2, *vol. per cent*	2.84	2.76	5.60	4.46	4.38	8.84	+1.62	+1.62	+3.24
Free O_2 *mM per l. blood*			0.10			0.05			−0.05
Free O_2, *vol. per cent*			0.23			0.12			−0.11
Combined O_2, *mM per l. blood*			9.48			7.44			−2.04
Combined O_2, *vol. per cent* ..			21.25			16.66			−4.59
Total O_2, *mM per l. blood* ...			9.58			7.49			−2.09
Total O_2, *vol. per cent*			21.48			16.78			−4.70
pCO_2, *mm. Hg*			7.3			14.7			+7.4
pO_2, *mm. Hg*			93.0			46.0			−47.0
Volume, *cc. per l. blood*	505.7	494.3	1,000.0	501.0	499.0	1,000.0	−4.7	+4.7	
pH	7.140	7.075		7.028	6.955		−0.112	−0.120	
r_{Cl}			0.827			0.870			+0.043
r_{HCO_3}			1.33			1.31			−0.02

TABLE 73.

Serum in diabetic coma.

		Arterial	Venous	Δ
H_2O	*cc. per l. serum*	924.8	924.1	−0.7
B	*mM* " " "	(150.4)	(151.5)	(+1.1)
X	" " " "	(31.2)	(34.6)	−3.4
Cl	" " " "	106.6	103.5	−3.1
BP	" " " "	10.3	9.9	−0.4
$BHCO_3$	" " " "	2.30	3.50	+1.20
H_2CO_3	" " " "	0.22	0.43	+0.21
Total CO_2	" " " "	2.52	3.93	+1.41

TABLE 74.

Cells in diabetic coma.

		Arterial	Venous	Δ
H_2O	*cc. per l. cells*	710.7	713.4	+7.3
B	*mM* " " "	119.0	118.0	−1.0
X	" " " "	19.2	16.7	−2.5
Cl	" " " "	67.7	70.0	+2.3
BP	" " " "	47.2	40.8	−6.4
$BHCO_3$	" " " "	2.33	3.59	+1.26
H_2CO_3	" " " "	0.16	0.34	+0.18
Total CO_2	" " " "	2.49	3.93	+1.44
Combined O_2	" " " "	19.2	15.0	−4.2
Total Hb	" " " "	19.9	19.7	−0.2

TABLE 75.

Arterial serum of A.V.B. at rest and of diabetic coma.

		A.V.B.	Coma	Δ
H_2O	*cc. per l. serum*	943.3	924.8	−18.5
B	*mM* " " "	154.0	(150.4)	(−3.6)
X	" " " "	17.00	(31.2)	(+14.2)
Cl	" " " "	99.32	106.6	+7.3
BP	" " " "	13.13	10.3	−2.8
$BHCO_3$	" " " "	24.55	2.30	−22.2
H_2CO_3	" " " "	1.22	0.22	−1.0
Total CO_2	" " " "	25.77	2.52	−23.2
pH		7.425	7.140	−0.285
Protein	*gm. per l. serum*	72.5	64.7	−7.8

TABLE 76.

Arterial cells of A.V.B. at rest and of diabetic coma.

		A.V.B.	Coma	Δ
H_2O	*cc. per l. cells*	705.0	710.7	+5.7
B	*mM* " " "	133.75	119.0	−14.7
X	" " " "	17.73	19.2	+1.5
Cl	" " " "	45.27	67.7	+22.4
BP	" " " "	56.50	47.2	−9.3
$BHCO_3$	" " " "	14.25	2.33	−11.9
H_2CO_3	" " " "	0.93	0.16	−0.77
Total CO_2	" " " "	15.18	2.49	−12.7
Combined O_2	" " " "	21.43	19.2	−2.2
Total Hb	" " " "	22.33	19.9	−2.4
pH		7.124	7.075	−0.049

Figure 202 is the nomogram for the blood of a case of diabetic coma. This figure yields tables 72 to 76. It is plain that serious discrepancies exist in the estimates of buffer action for this case. The errors are probably due not so much to experiment as to ignorance of conditions within the cell. In most respects, however, the nomogram and figures for this case yield sufficiently accurate information.

The effect on the acid-base equilibrium of the blood

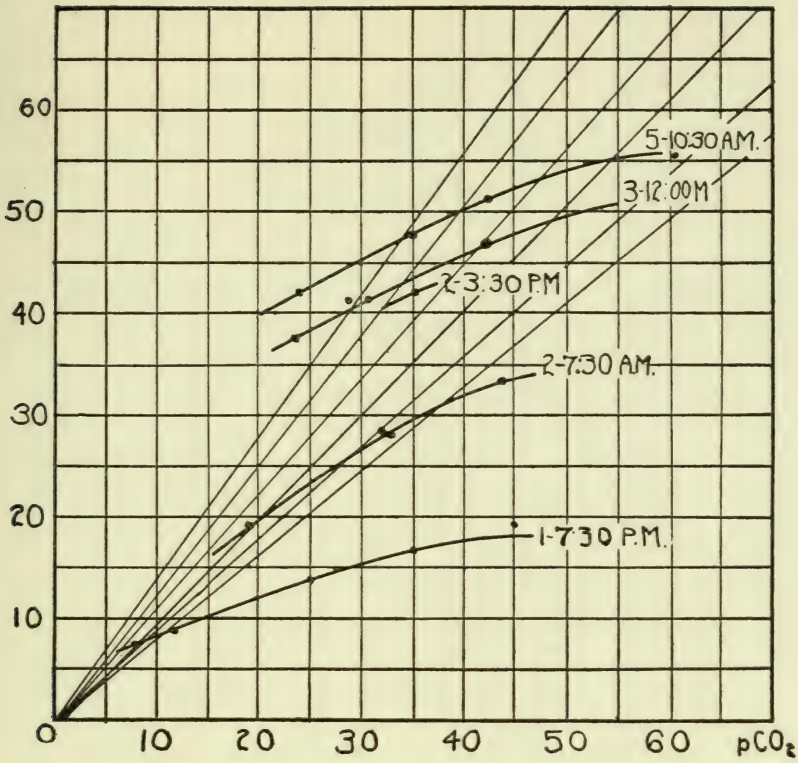

Fig. 203

Diabetic Coma: Effect of Insulin on Blood Carbonic Acid

produced by administration of insulin in a similar case is illustrated by figure 203 taken from the paper of Bock, Field, and Adair.[135]

Finally, figure 204 gives the nomogram for a case of congenital malformation of the heart with deep cyanosis and polycythemia.[136]

[135] Bock, Field, and Adair, *Journal of Metabolic Research,* IV, 27 (1923).

[136] L. J. Henderson, *Comptes rendus de l'Académie des Sciences,* CLXXX, 2066 (1925).

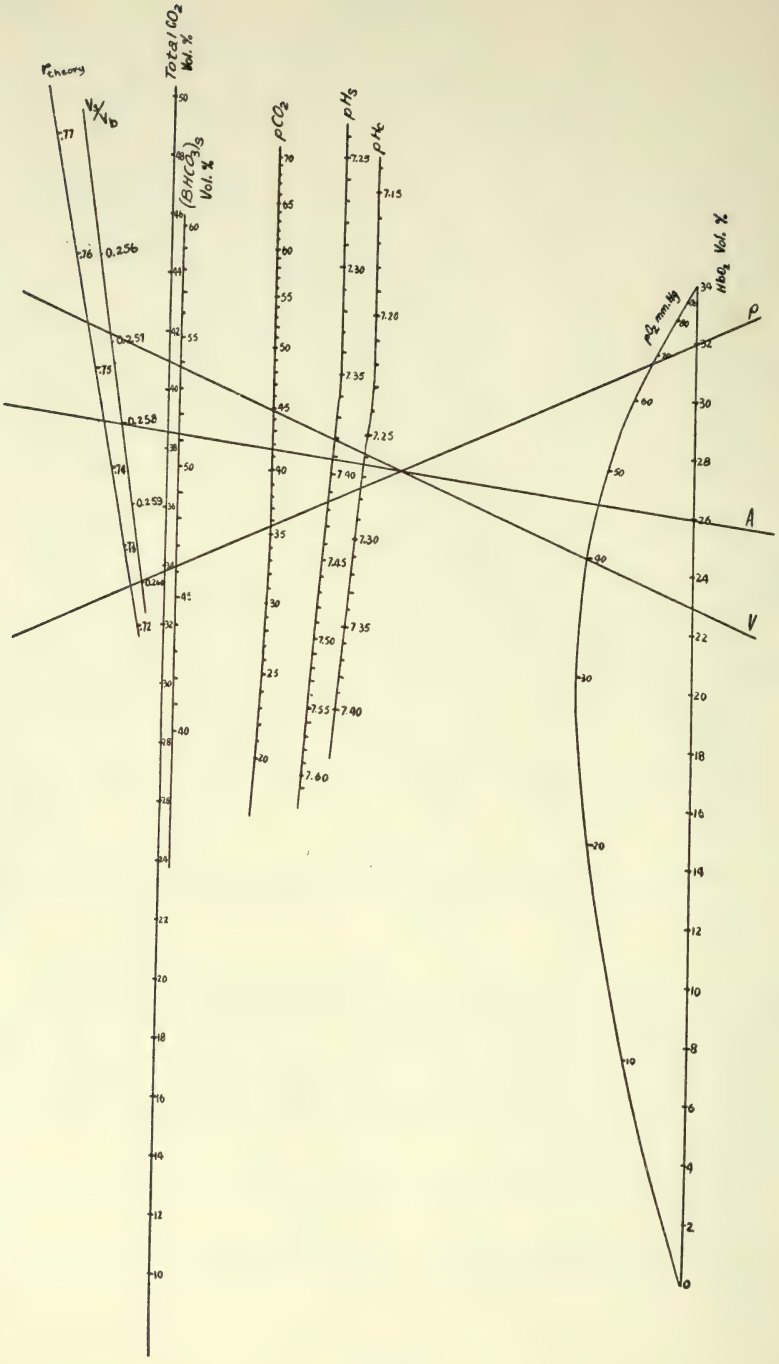

Fig. 204

Blood in a Case of Congenital Malformation of the Heart

CHAPTER XI

OTHER SPECIES

AFTER the study of human blood we shall now consider the blood of other species. Of this subject our knowledge is but fragmentary; first, because the British and American schools of physiology have followed the example of Haldane and formed the habit of experimenting on themselves; secondly, because it is in general difficult, or even impossible, to make a correct diagnosis of the physiological state of a lower animal; and, finally, because the animal cannot either obey the instructions of the experimenter or impart information.

A complete nomogram of horse blood was made by Van Slyke, Wu, and McLean.[137] A transformation of this nomogram according to the method of construction employed in this book is given in figure 205.

The positions of the arterial and venous blood lines on this chart are arbitrarily chosen, and, in particular, the correct position of the latter line is very uncertain. From the figure, table 77 has been constructed. In studying this table it must, therefore, be remembered that, although the composition of the blood has been accurately determined, the estimates of the respiratory cycle are arbitrary.

From the standpoint adopted in working out our approximate description of the conditions, the composition of horse blood is almost indistinguishable from that of human blood. In fact the only difference great enough to attract attention is in the concentration of hemoglobin within the cells, or, to speak in terms of actual measurements, in the oxygen capacity per liter of cells. If we may

[137] Van Slyke, Wu, and McLean, *Journal of Biological Chemistry,* LVI, 784 (1923).

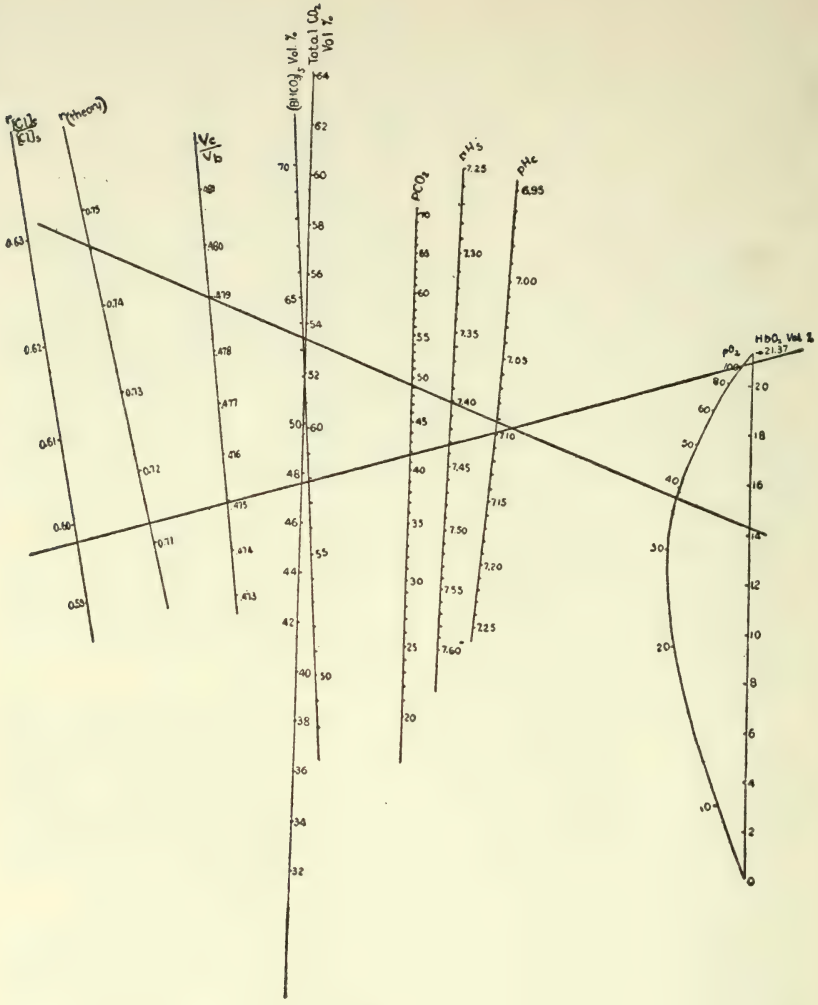

Fig. 205

Blood of Horse

TABLE 77.

Estimation of the changes occurring in the normal respiratory cycle of the horse.

	ARTERIAL			VENOUS			CHANGES		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
(Hb) *mM* per kg. blood		9.0	9.0		9.0	9.0			
$(P)_s$ gm. " " "	37.0		37.0	37.0		37.0			
(B) *m.-Eq.* " " "	73.0	54.0	127.0	73.0	54.0	127.0			
(H_2O) *kg.* " " "	0.464	0.316	0.78	0.460	0.320	0.780	−0.004	+0.004	
(Cl) *m.-Eq.* " " "	51.2	24.8	76.0	50.0	26.0	76.0	−1.1	+1.1	
(BP) " " " "	6.62	22.01	28.63	6.54	19.87	26.41	−0.08	−2.14	−2.22
($BHCO_3$) " " " "	12.79	6.21	19.00	13.96	7.26	21.22	+1.17	−1.05	+2.22
(H_2CO_3) " " " "	0.626	0.426	1.052	0.733	0.510	1.243	+0.11	+0.08	+0.19
Total CO_2 " " " "	13.42	6.63	20.05	14.69	7.77	22.46	+1.27	+1.14	+2.41
Combined O_2 *mM* " " "		8.8	8.8		6.0	6.0		−2.8	−2.8
Dissolved O_2 " " " "			0.14			0.03			−0.1
Total O_2 " " " "			8.94			6.03			−2.9
pCO_2 *mm. Hg* " " "	41.6	41.6	41.6	49.2	49.2	49.2	+7.6	+7.6	+7.6
pO_2 " " " " "	100.0	100.0	100.0	38.0	38.0	38.0	−62.0	−62.0	−62.0
pH	7.43	7.283		7.40	7.272		−0.030	−0.011	
Volume l., *per l. blood*	0.525	0.475	1.00	0.521	0.479	1.000	−0.004	+0.004	
r ratio			0.713			0.746			+0.033
(*r*) ratio			0.485			0.519			+0.034
Respiratory quotient $\frac{\Delta CO_2}{\Delta O_2}$ ...									0.83

draw a conclusion from these observations, human cells seem to have a capacity slightly greater than that of horse cells to transport oxygen. This may be due to differences between the hemoglobins of the two species or to differences of the physico-chemical structure of the red cells in man and the horse. Both differences no doubt exist. But, since greater variations than those in question occur in man under pathological conditions, and the state of the horse is not accurately known, the interpretation of the facts must remain doubtful.

On general principles, especially from consideration of the principle of similitude, it is not to be expected that mere differences in size should lead directly to differences in the physico-chemical composition of the blood of similar species. Nevertheless, it is at least worthy of note that, in this case at least, such differences have not arisen indirectly. The fact is significant of the very general character of the factors that determine the constitution and properties of blood.

The blood of the snapping turtle, *Chelydra serpentina,* has been studied by Redfield and Southworth,[138] and by my collaborators at the Massachusetts General Hospital. The results are printed as a nomogram in figure 206. The facts concerning the respiratory cycle and the composition of the blood of this animal are also given in the usual manner in tables 78 to 80.

[138] Redfield and Southworth, *Journal of General Physiology,* IX, 387 (1926).

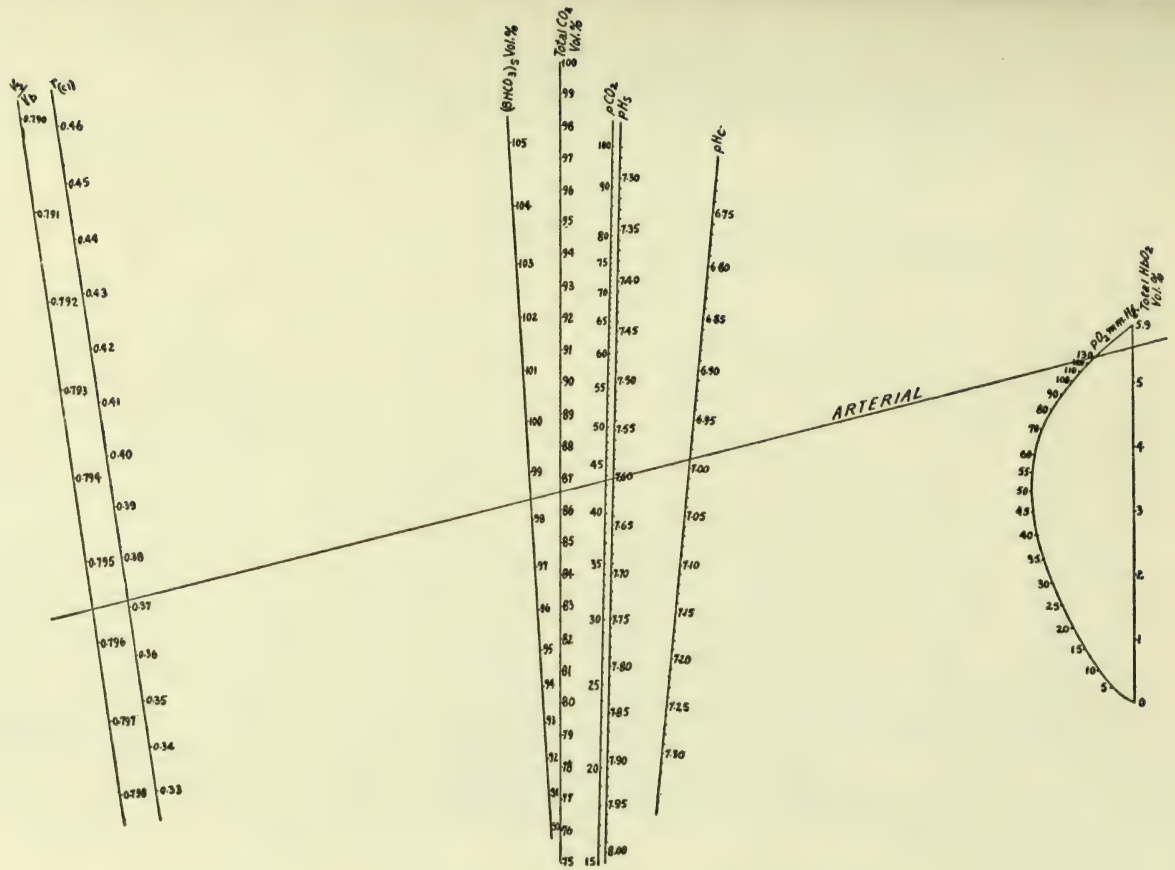

Fig. 206

Blood of Snapping Turtle: Temperature 37.5°

TABLE 78.

Blood of the snapping turtle (Chelydra serpentina) at 37.5°.

Concentration of hemoglobin = 2.63 *mM* per liter of blood.
Concentration of serum proteins = 40.8 gm. per liter of blood.

	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	760.0	148.0	908
B *mM* " " "	116.50	20.60	137.10
X " " " "	11.65	0.85	12.50
Cl " " " "	62.11	4.49	66.60
BP " " " "	7.78	12.86	20.64
$BHCO_3$ " " " "	34.96	2.40	37.36
$BHCO_3$, *vol. per cent*	78.32	5.38	83.70
H_2CO_3, *mM per l. blood*	1.07	0.21	1.28
H_2CO_3, *vol. per cent*	2.40	0.47	2.87
Total CO_2, *mM per l. blood*	36.03	2.61	38.64
Total CO_2, *vol. per cent*	80.72	5.85	86.57
Free O_2, *mM per l. blood*			0.15
Free O_2, *vol. per cent*			0.32
Combined O_2, *mM per l. blood*			2.50
Combined O_2, *vol. per cent*			5.60
Total O_2, *mM per l. blood*			2.65
Total O_2, *vol. per cent*			5.92
pCO_2, *mm. Hg*			43.5
pO_2, *mm. Hg*			130
Volume, *cc. per l. blood*	795.6	204.4	1,000
pH	7.60	6.99	
r_{Cl}			0.371
r_{HCO_3}			0.353

TABLE 79.

Arterial serum of the snapping turtle at 37.5°.

H_2O *cc. per l.*	955.2
B *mM* " "	146.50
X " " "	14.65
Cl " " "	78.11
BP " " "	9.78
$BHCO_3$ " " "	43.96
H_2CO_3 " " "	1.35
Total CO_2 " " "	45.31

TABLE 80.

Arterial cells of the snapping turtle at 37.5°.

H_2O	*cc. per l.*	724.3
B	*mM* " "	100.70
X	" " "	4.15
Cl	" " "	21.90
BP	" " "	62.92
$BHCO_3$	" " "	11.73
H_2CO_3	" " "	1.03
Total CO_2	" " "	12.76
Combined O_2	" " "	12.2
Total Hb	" " "	12.9

We are here concerned with a vertebrate which differs widely from the mammals in both morphological and physiological characters. Morphologically, the difference that has the greatest influence upon the properties of the blood is perhaps the structure of the heart. As a result of communication between the two sides of the heart in this animal, there appear to be, in addition to the local venous bloods, three principal kinds of blood: mixed venous blood, arterial blood, and a mixture of these which returns to the lungs from the heart. These facts are represented on the nomogram and in the tables. We shall consider them in the next chapter. Physiologically, the most striking peculiarity of the turtle in contrast to the mammals is, no doubt, the variable and generally low temperature of the body. This is associated with a low metabolic rate and with the transport of small quantities of oxygen and of carbonic acid. In turn, these peculiarities are associated with marked differences in physico-chemical properties from the mammalian type of blood. The facts are all clearly presented in the nomogram and tables and at this stage of our study require no further elucidation.

But variable body temperature introduces a complication that has not heretofore arisen and, in this instance, we must take account of the effect of temperature upon the properties of the physico-chemical system of the

blood. In mammals it is possible to neglect, without very erroneous conclusions, the effects of the fluctuation of temperature which may occur in the normal individual, and even in fever such changes may not be great, but here the fluctuation of temperature may amount to many degrees.

Needless to say, temperature is one of the most familiar and one of the most important of physico-chemical variables. Therefore, if blood were a simple system, there would be little difficulty in treating the question from a theoretical standpoint. Indeed, this may be done for certain aspects of the problem, and I did in fact many years ago thus discuss the variations of hydrogen ion concentration that accompany variations of temperature in human blood.[139] But it would be at present a hopeless task to treat theoretically the variation of a nomogram with variation of temperature.

Accordingly, we have undertaken an experimental study of the question and have constructed a nomogram, figure 207, representing the properties of turtle blood at 20 degrees. Tables 81, 82, and 83 are constructed from this figure. Figure 206 above and tables 78 to 80 describe the

[139] L. J. Henderson, *Ergebnisse der Physiologie, Jahrgang* VIII, p. 301, 1909.

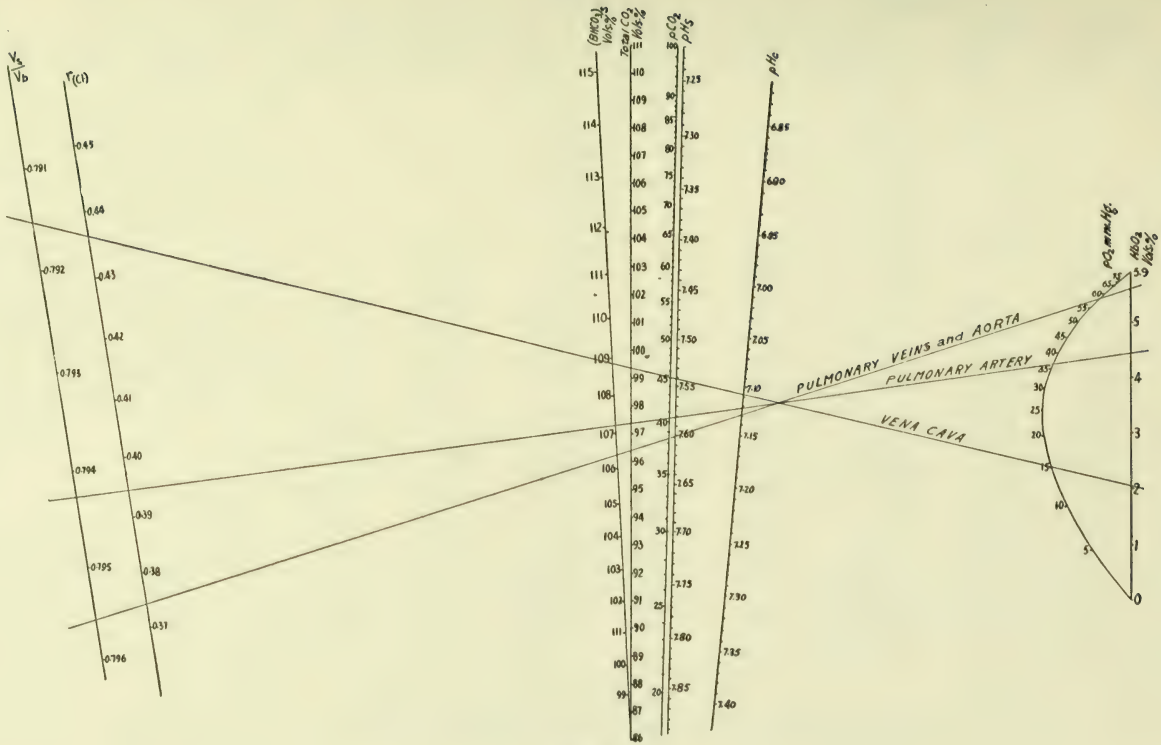

Fig. 207

Blood of Snapping Turtle: Temperature 20°

TABLE 81.

Blood of the snapping turtle (Chelydra serpentina) at 20°.

Concentration of hemoglobin = 2.63 *mM* per liter of blood.
Concentration of serum proteins = 40.8 gm. per liter of blood.
Respiratory quotient = 0.80.

	Blood from lungs			Blood from tissues			Blood entering lungs		
	Serum	Cells	Whole blood	Serum	Cells	Whole blood	Serum	Cells	Whole blood
H_2O *cc. per l. blood*	760.0	148.0	908.0	755.9	152.1	908.0	758.7	149.3	908.0
B *mM* " " "	116.50	20.60	137.10	116.50	20.60	137.10	116.50	20.60	137.10
X " " " "	8.84	0.50	9.34	9.23	0.60	9.83	8.96	0.60	9.56
Cl " " " "	62.08	4.52	66.60	61.23	5.37	66.60	61.79	4.81	66.60
BP " " " "	7.78	12.04	19.82	7.62	10.69	18.31	7.73	11.52	19.25
$BHCO_3$ " " " "	37.80	3.54	41.34	38.42	3.94	42.36	38.02	3.67	41.69
$BHCO_3$, *vol. per cent*	84.65	7.94	92.59	86.06	8.83	94.89	85.16	8.22	93.38
H_2CO_3, *mM per l. blood*	1.44	0.28	1.72	1.68	0.33	2.01	1.50	0.29	1.79
H_2CO_3, *vol. per cent*	3.22	0.62	3.84	3.75	0.75	4.50	3.36	0.66	4.02
Total CO_2, *mM per l. blood*	39.24	3.82	43.06	40.10	4.27	44.37	39.52	3.96	43.48
Total CO_2, *vol. per cent*	87.87	8.56	96.43	89.81	9.58	99.39	88.52	8.88	97.40
Combined O_2, *mM per l. blood*		2.50	2.50		0.92	0.92		1.98	1.98
Combined O_2, *vol. per cent*		5.60	5.60		2.07	2.07		4.43	4.43
Free O_2, *mM per l. blood*			0.10			0.02			0.06
Free O_2, *vol. per cent*			0.22			0.05			0.14
Total O_2, *mM per l. blood*			2.60			0.94			2.24
Total O_2, *vol. per cent*			5.82			2.12			4.57
pCO_2, *mm. Hg*			38.5			45.2			40.4
pO_2, *mm. Hg*			57.0			15.0			37.0
Volume, *cc. per l. blood*	795.6	204.4	1,000.0	791.5	208.5	1,000.0	794.3	205.7	1,000.0
pH	7.600	7.128		7.541	7.107		7.581	7.121	
r_{HCO_3}			0.482			0.509			0.491
r_{Cl}			0.374			0.436			0.395

TABLE 82.

Serum of turtle at 20°.

		From lungs	From vena cava	To lungs
H_2O	*cc. per l. serum*	955.2	954.8	955.0
B	*mM* " " "	146.50	147.20	146.70
X	" " " "	11.07	11.66	11.27
Cl	" " " "	78.05	77.36	77.83
BP	" " " "	9.78	9.63	9.73
$BHCO_3$	" " " "	47.50	48.55	47.87
H_2CO_3	" " " "	1.80	2.12	1.89
Total CO_2	" " " "	49.30	50.67	49.76

TABLE 83.

Cells of turtle at 20°.

		From lungs	From vena cava	To lungs
H_2O	*cc. per l. cells*	724.3	729.3	725.8
B	*mM* " " "	100.7	98.8	100.1
X	" " " "	2.4	2.9	2.9
Cl	" " " "	22.1	25.8	23.4
BP	" " " "	58.9	51.2	56.0
$BHCO_3$	" " " "	17.3	18.9	17.8
H_2CO_3	" " " "	1.3	1.6	1.4
Total CO_2	" " " "	18.6	20.5	19.2
Combined O_2	" " " "	12.2	4.4	9.6
Total Hb	" " " "	12.9	12.6	12.8

properties of the same blood removed from the body of the turtle under ordinary conditions and then brought to a temperature of 37.5 degrees, so that the conditions shall be similar to those in our studies of mammalian blood. A comparison of the figures and tables will yield information concerning the variations that occur in the same specimens of blood when the temperature is varied.

Such an experiment is, however, very far from what is required to determine the changes in the blood of an organism when the temperature of the whole body is varied. Thus far we have not undertaken a study of this question.

But Austin, Sunderman, and Carnack,[140] in their studies of the blood of the alligator, have presented observations on the composition of blood serum drawn from the same individual before and after varying the temperature of the body. These measurements prove that variations of body temperature may be accompanied by considerable variations in the masses of the components of blood as well as by those changes, dependent upon variations of the solubility of gases and of chemical affinities, to which the variations in our two turtle blood nomograms are to be ascribed. The measurements of Austin are not sufficiently extensive to enable us to analyze the phenomenon in detail; but they show that change of temperature in an organism is not less important than those physiological and pathological variations which we have studied in this book. On thermodynamical grounds the fact is not difficult to understand, and its biological implications are manifest.

In the above discussion specific differences in hemoglobins have been disregarded. Such differences, however, exist[141] and must be taken into account as comparative studies are extended.

In many invertebrates blood contains no cells that take part in the respiratory activity and in such forms the copper protein compound, hemocyanin, is far more common than hemoglobin as the oxygen carrier. The properties of several varieties of such blood have been carefully studied by Redfield and his associates as well as by other investigators.[142] In the absence of cells the system may be regarded as possessing but a single phase and, under these circumstances, it is possible, in a first approximation, to neglect the presence of chlorides. Thus the com-

[140] Austin, Sunderman, and Carnack, *Journal of Biological Chemistry,* LXXII, 677 (1927).

[141] Hastings, Van Slyke, Neill, Heidelberger, and Harington, *Journal of Biological Chemistry,* LX, 89 (1924).

[142] *Cf.* Redfield, Coolidge, and Hurd, *Journal of Biological Chemistry,* LXIX, 475 (1926).

ponents of the system may be taken as five: water, carbon dioxide, oxygen, base, and protein. The absence of cells obviates all the complications of treatment associated with the heterogeneous equilibrium and the Gibbs-Donnan law. Otherwise the chief differences in this case depend upon the differences in properties of the various hemocyanins and of the hemoglobins.

The hemocyanins of different species appear to differ widely in physico-chemical properties, though they are perhaps all alike in combining with oxygen in the proportion of two atoms of copper to one molecule of oxygen. These compounds, like oxyhemoglobin, are dissociable and the equilibrium between oxygen and hemocyanin may be represented by curves which are unmistakably similar in character to oxyhemoglobin dissociation curves. The affinities for oxygen are very variable from species to species and this variation seems to be an adaptation to the conditions of life similar to that observed by Krogh and Leitch[143] in certain species of fishes. In this respect, the contrast is great between the horseshoe crab, *Limulus polyphemus,* and the squid, *Loligo pealii.* The horseshoe crab is a bottom form which often half buries itself when in shallow water so that it may be uncovered at low tide. Under these circumstances the partial pressure of oxygen probably falls very low. The squid is a pelagic form which, so far as known, never leaves an environment where the partial pressure of oxygen is close to that of the atmosphere. Also the horseshoe crab is inactive, the squid one of the most active of marine invertebrates. Figures 208 and 209 give Redfield's oxygen dissociation curve of the bloods of these two forms and clearly show that in the horseshoe crab a supply of oxygen at low pressure, corresponding to the inactive existence in the absence of an ample reserve, is afforded. In the squid the supply is at a much higher pressure and this is more suitable to an active existence, but it greatly increases the risk of anoxemia.

[143] Krogh and Leitch, *Journal of Physiology,* LII, 288 (1919).

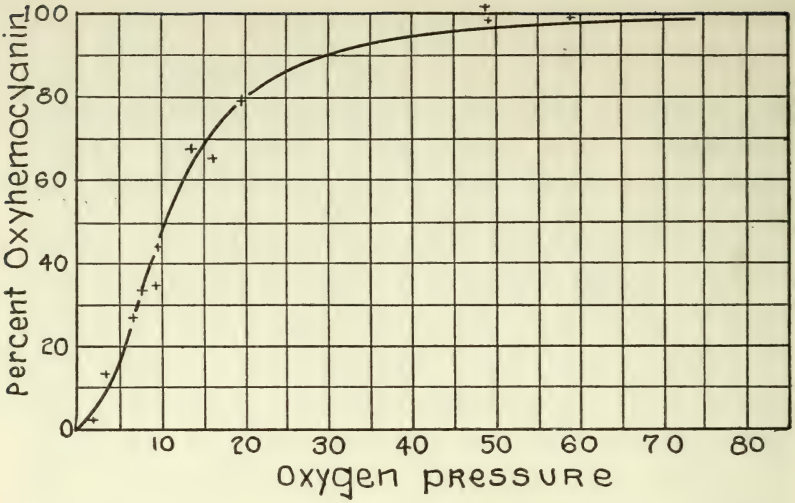

Fig. 208

Oxygen Dissociation Curve: Blood of Horseshoe Crab

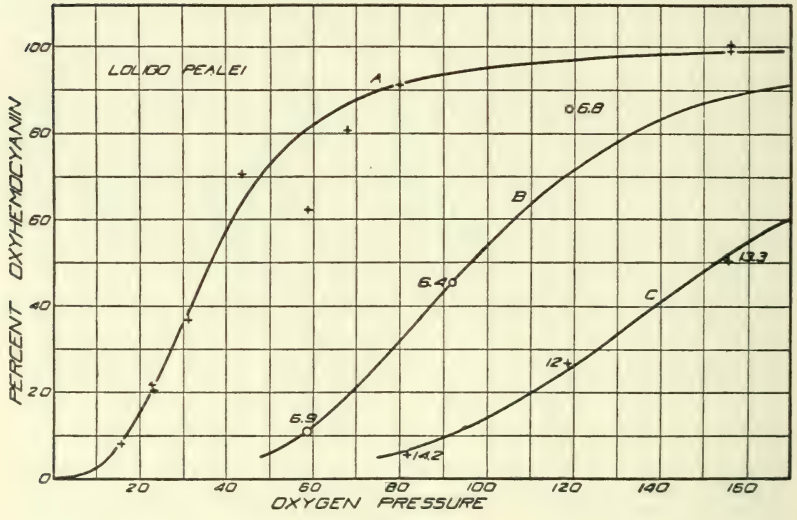

Fig. 209

Oxygen Dissociation Curves: Blood of Squid

The studies of Parsons and Parsons,[144] of Collip[145] and of Redfield and his associates[146] show that hemocyanin is the principal factor beside carbonic acid and base in the transport of carbon dioxide in the blood of these forms of animals. Therefore, it is possible once more to represent the conditions approximately by the simple reaction

$$BP + H_2CO_3 = BHCO_3 + HP.$$

Redfield's carbon dioxide dissociation curves for the blood of the horseshoe crab, of the squid, and of a marine gastropod, *Busycon canaliculatum,* are given in figure 210.

In the squid, variation in partial pressure of carbon dioxide is accompanied by a change in equilibrium between oxygen and hemocyanin and this change is similar to that of the equilibrium between oxygen and hemoglobin in man. The facts are represented in the customary manner on Redfield's dissociation curve, figure 209. He has discussed them as follows: "Not only does the addition of carbon dioxide diminish the affinity of this blood for oxygen, thus shifting the curves to the right, but it does so in a degree unequalled in any other blood concerning which we have knowledge. An examination of these curves makes it clear why squid blood becomes colorless when shaken with alveolar air. We have observed that six per cent of carbon dioxide will decolorize this blood even in the presence of an atmosphere of oxygen. *Loligo* blood is well buffered at low carbon dioxide tensions, as we shall show. The magnitude of the carbon dioxide effect consequently cannot be attributed to any exceptional action of carbonic acid in changing the hydrogen ion concentration of the blood. Rather it must be due to a characteristic property of the *Loligo* hemocyanin itself."

[144] Parsons and Parsons, *Journal of General Physiology,* VI, 153 (1923-1924).

[145] J. B. Collip, *Journal of Biological Chemistry,* XLV, 23 (1920-1921).

[146] Redfield, Coolidge, and Hurd, *Journal of Biological Chemistry,* LXIX, 475 (1926).

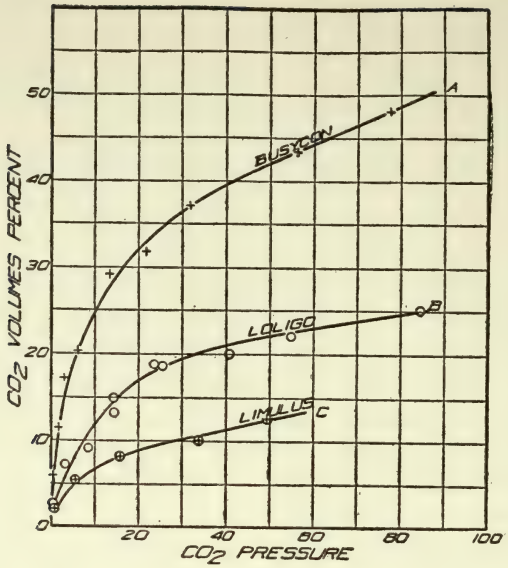

Fig. 210

Carbon Dioxide Dissociation Curves: Bloods of Horseshoe Crab, Squid, and Busycon

Long ago it was suggested by Rona and Ylppö[147] that the carbonic acid effect upon hemoglobin might be reversed in acid solutions and recently the fact has been observed at the Harvard Medical School by Ferry and Green. In *Limulus,* however, and in *Busycon,* even under normal conditions increase of pressure of carbon dioxide is accompanied by increase of affinity of hemoglobin for oxygen. The observations of Redfield, Coolidge, and Hurd on *Busycon* are given in figure 211.

The reciprocal effect of oxygen on carbonic acid equilibrium has also been studied by Redfield, Coolidge, and Hurd. This is shown in figures 212 and 213.

The peculiarities of the hemocyanins that are here in

[147] Rona and Ylppö, *Biochemische Zeitschrift,* LXXVI, 187 (1916).

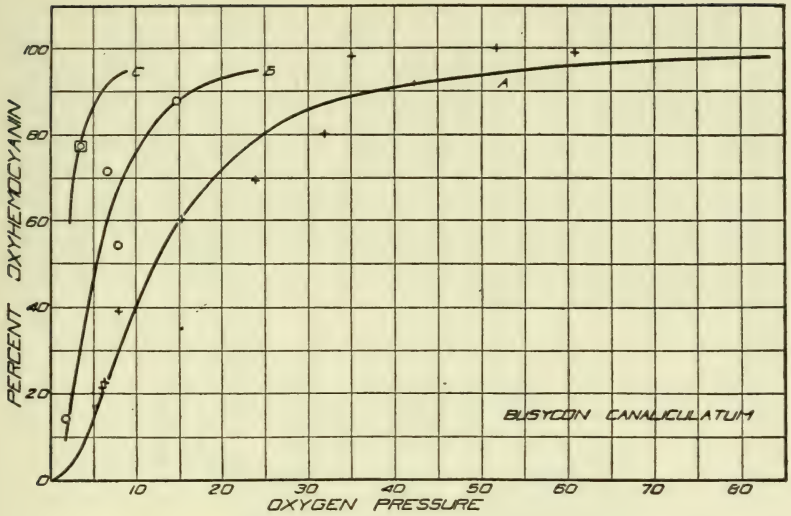

Fig. 211

Oxygen Dissociation Curves: Busycon

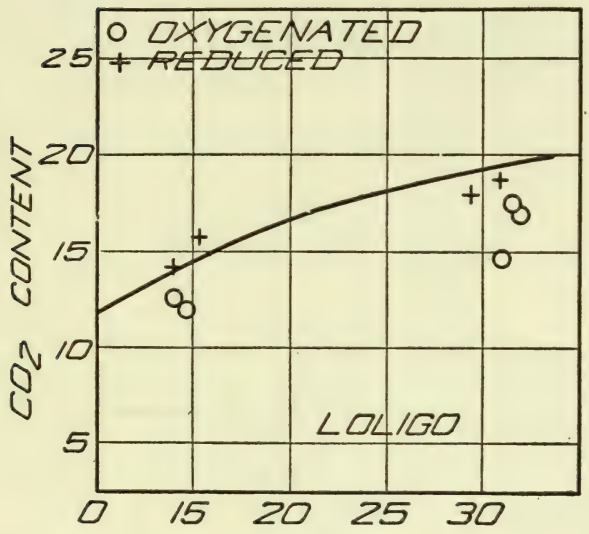

Fig. 212

The Oxygen Effect in Squid Blood

question may perhaps lead to an explanation of that chemical problem, which in the case of hemoglobin, has long baffled all investigators. Redfield has already succeeded in showing the dependence of the variation of affinity for oxygen with hydrogen ion concentration upon the acid and basic properties of the several molecular species of hemocyanin and of hemoglobin. His results are given in figure 214. From this point it can hardly be a long step to the complete theoretical elucidation of oxygen dissociation curves.

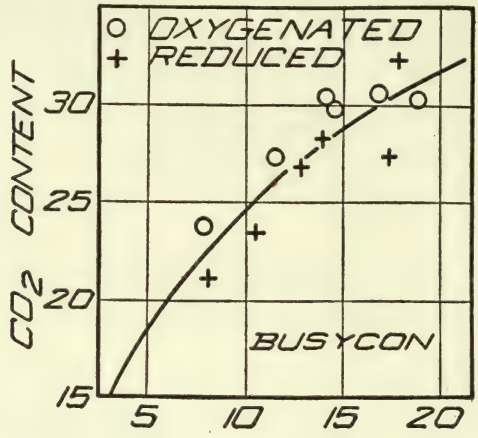

Fig. 213

The Oxygen Effect in Busycon *Blood*

In these chapters, it has not been our aim to carry the theoretical physico-chemical treatment beyond the point judged necessary for the interpretation of the physiological facts. We may, accordingly, pass over this interesting chemical problem and turn to the description of the whole physico-chemical system of these types of blood.

Figure 215 gives Redfield's approximate estimate of the properties of the blood of *Busycon* for which the usual

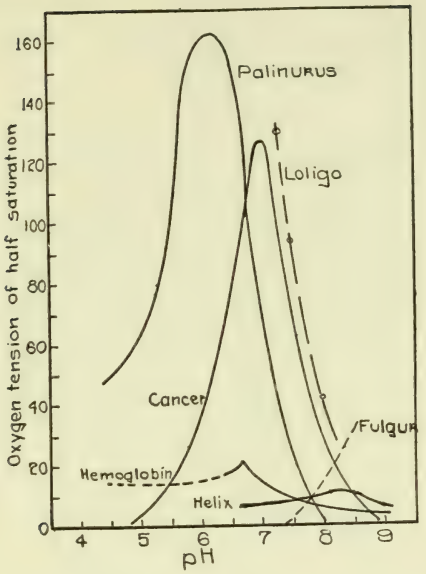

Fig. 214

Oxygen Affinity and Acid-Base Properties of Hemocyanins and Hemoglobin

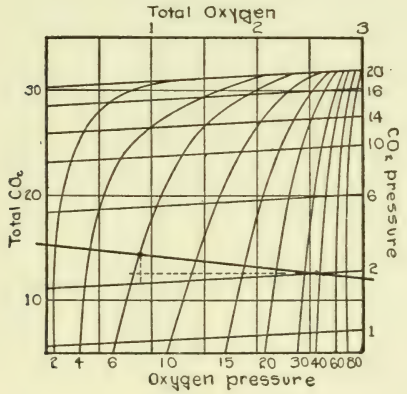

Fig. 215

Blood of Busycon

carbonic acid effect is reversed. Figure 216 presents a more exact description of the blood of *Loligo*. Tables 84 and 85 complete the description.

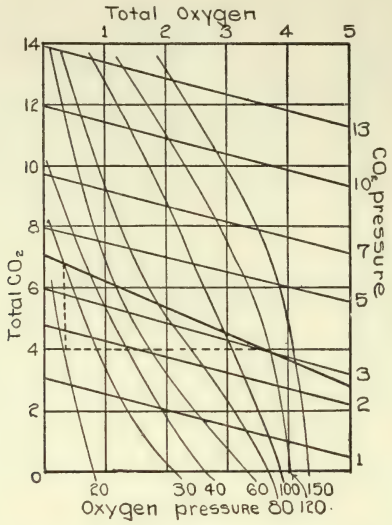

Fig. 216

Blood of Loligo

TABLE 84.

Busycon.

	A	V	Δ
$BHCO_3$, *mM per l.*	5.61	6.21	+0.60
$BHCO_3$, *vol. per cent*	12.5	13.9	+1.4
H_2CO_3, *mM per l.*	0.11	0.18	+0.07
H_2CO_3, *vol. per cent*	0.25	0.40	+0.15
Total CO_2, *mM per l.*	5.72	6.39	+0.67
Total CO_2, *vol. per cent*	12.8	14.3	+1.5
Free O_2, *mM per l.*	0.07	0.01	−0.06
Free O_2, *vol. per cent*	0.16	0.02	−0.14
Combined O_2, *mM per l.*	1.09	0.39	−0.70
Combined O_2, *vol. per cent*	2.44	0.88	−1.56
Total O_2, *mM per l.*	1.16	0.40	−0.76
Total O_2, *vol. per cent*	2.6	0.9	−1.7
pCO_2, *mm. Hg*	2.0	3.3	+1.3
pO_2, *mm. Hg*	36	6	−30
pH	7.966	7.793	−0.173

TABLE 85.

Loligo.

	A	V	Δ
$BHCO_3$, *mM per l.*	1.62	2.82	+1.20
$BHCO_3$, *vol. per cent*	3.63	6.32	+2.69
H_2CO_3, *mM per l.*	0.16	0.22	+0.06
H_2CO_3, *vol. per cent*	0.36	0.49	+0.13
Total CO_2, *mM per l.*	1.79	3.04	+1.25
Total CO_2, *vol. per cent*	4.0	6.8	+2.8
Free O_2, *mM per l.*	0.23	0.06	−0.17
Free O_2, *vol. per cent*	0.51	0.13	−0.38
Combined O_2, *mM per l.*	1.33	0.07	−1.26
Combined O_2, *vol. per cent*	2.98	0.16	−2.82
Total O_2, *mM per l.*	1.56	0.13	−1.43
Total O_2, *vol. per cent*	3.5	0.3	−3.2
pCO_2, *mm. Hg.*	3	4	+1
pO_2, *mm. Hg*	115	30	−85
pH	7.251	7.365	+0.114

The facts here presented are too few and too fragmentary to enable us to survey the extensive problem of the blood of lower organisms. Yet it is already evident that existing methods are, on the whole, sufficient for the purpose. The study of human blood has overcome most of the difficulties and the opportunity for a rational treatment of the blood of other forms is open to any skilful experimenter.

CHAPTER XII

CIRCULATORY ADAPTATIONS

THE variations in the composition of the blood described in the three preceding chapters are associated with variations in the circulation and in other physiological processes. In Chapter VIII the nature of such interrelations was discussed from a theoretical standpoint, and it now remains to study the facts which bear upon this question. A long series of experiments by Bock, Field, Dill,[148] and others at the Massachusetts General Hospital and more recently in the Harvard Fatigue Laboratory will enable us to introduce certain novel considerations into the discussion. But the treatment must at best be fragmentary for, on the whole, this subject has been surveyed less completely than that of the comparative physiology of blood.

We have seen that the rate of blood flow (BF measured in liters per minute) is a function of the composition of the blood, of the metabolic rate, and of many other variables. Among these is posture, and with this we may begin our exposition. It is well known that variations of venous pressure in an extremity are accompanied by changes in the composition of the blood and in the rate of its return to the heart. The experiments of Bock and Field, of Miss Turner,[149] and of Thompson, Thompson, and Dailey[150a]

[148] Bock and Field, *Journal of Biological Chemistry,* LXII, 269 (1924-1925); Dill, Hurxthal, van Caulaert, Fölling, and Bock, *Journal of Biological Chemistry,* LXXIV, 303 (1927); Dill, Lawrence, Hurxthal, and Bock, *Journal of Biological Chemistry,* LXXIV, 313 (1927); and unpublished work.

[149] A. H. Turner, *American Journal of Physiology,* LXXX, 601 (1927).

[150a] Thompson, Thompson, and Dailey, *Proceedings National Academy of Sciences,* XIV, 94 (1928).

have added the proof that changes in posture produce similar effects. A mere change from the recumbent to the sitting position is accompanied by marked diminution in blood flow, while change from the recumbent position to the standing still position may reduce the blood flow one-half, say from eight to four liters per minute. This effect is much diminished by even slight movements of the legs, and is at least partly dependent upon the effect of gravity on venous pressure in the lower portions of the body and on the consequent diminution of the "venous return" to the heart. When the standing still position is carefully maintained for more than a few minutes, the legs swell and a marked feeling of distress arises. It is rare that the position can be maintained for 30 minutes before fainting ends the experiment. The observations of Thompson suggest that the effect of gravity extends to the capillaries and prove that, under these conditions, the composition of the blood changes greatly.

Table 86 gives average values for the composition of the blood in the four subjects most completely studied by Thompson. The first row of figures describes the conditions of the blood while the subject is in the recumbent position, and the second row gives the corresponding values for the condition after nearly a half hour of standing still. At this point, the total blood plasma of the body has diminished by 290 cc. or nearly 12 per cent, while the total volume of cells in the blood has remained constant. Thus the whole volume of blood in the body has decreased about seven or eight per cent, while the concentration of red cells has risen about three per cent and the red count has also increased. Meanwhile, the plasma has become a more concentrated solution with an increase in protein concentration and a corresponding decrease in the concentration of water. Accompanying these variations is a rise of the specific gravity of the plasma.

These facts may be explained by the hypothesis that there has occurred a net loss of 290 cc. of protein free fluid from the blood with no other important change, a

TABLE 86.

Changes in blood while standing still.

	TOTAL VOLUME				Red count	PLASMA		
	Plasma cc.	Cells cc.	Blood cc.	Cell volume cc. per l. blood	millions per cu. mm.	Protein g. per 100 cc.	Water g. per 100 cc.	S.G.
Recumbent position ...	2,475	1,510	3,985	379	4.2	6.9	91.5	1.026
Upright position	2,185	1,505	3,690	408	4.7	8.1	90.6	1.028
Difference observed	−290	−5	−295	+29	+0.5	+1.2	−0.9	+0.002
Difference calculated ...			−290	+30	+0.33	+0.92	−1.1	+0.003
"Error"		−5	−5	−1	+0.17	+0.28	+0.2	−0.001

view which is supported by the swelling of the legs, by consideration of the probable effect of gravity, and by the fact that a half hour in the recumbent position suffices to reverse all changes.

When the state of the body changes, it is rare that the facts can be so simply interpreted and even muscular activity in normal subjects presents a far more intricate problem. The studies of this subject to which we shall now turn have to do with the physiological changes in several normal men while riding a stationary bicycle. The observations were all made when an approximately stationary state had been reached during the period of about 20 minutes following the first five minutes of uniform work.

The subjects of these experiments are four men: De Mar, the marathon runner, age, 39; height, 163 cm.; weight, 61.5 kg.; vital capacity, 5500 cc., who, for the past 20 years, has continuously maintained a state of physical training involving great muscular activity; and three persons of sedentary habits, D.B.D., age, 35; height, 180 cm.; weight, 71.5 kg.; vital capacity, 4700 cc.; A.V.B., age, 38; height, 177 cm.; weight, 71½ kg.; vital capacity, 4200 cc.; and C.V.C., age 25; height, 163 cm.; weight, 59 kg.; vital capacity, 3800 cc. All are healthy men, free from known disabilities, but they vary greatly in capacity to do muscular work and this capacity may perhaps be graded in the order in which the subjects are mentioned above as: Very great, great, small, and very small. This wide individual variation has been an advantage in the prosecution of the work.

The experiments on De Mar cover a period of two weeks, those on D.B.D. and A.V.B. six months, those on C.V.C. about six weeks. In the case of De Mar, the experiments were performed before breakfast, in the other cases, usually one to three hours after breakfast. There is a good deal of fluctuation in the experimental data which may be fairly attributed to uncontrolled, and perhaps uncontrollable, slight fluctuations in the state of the organ-

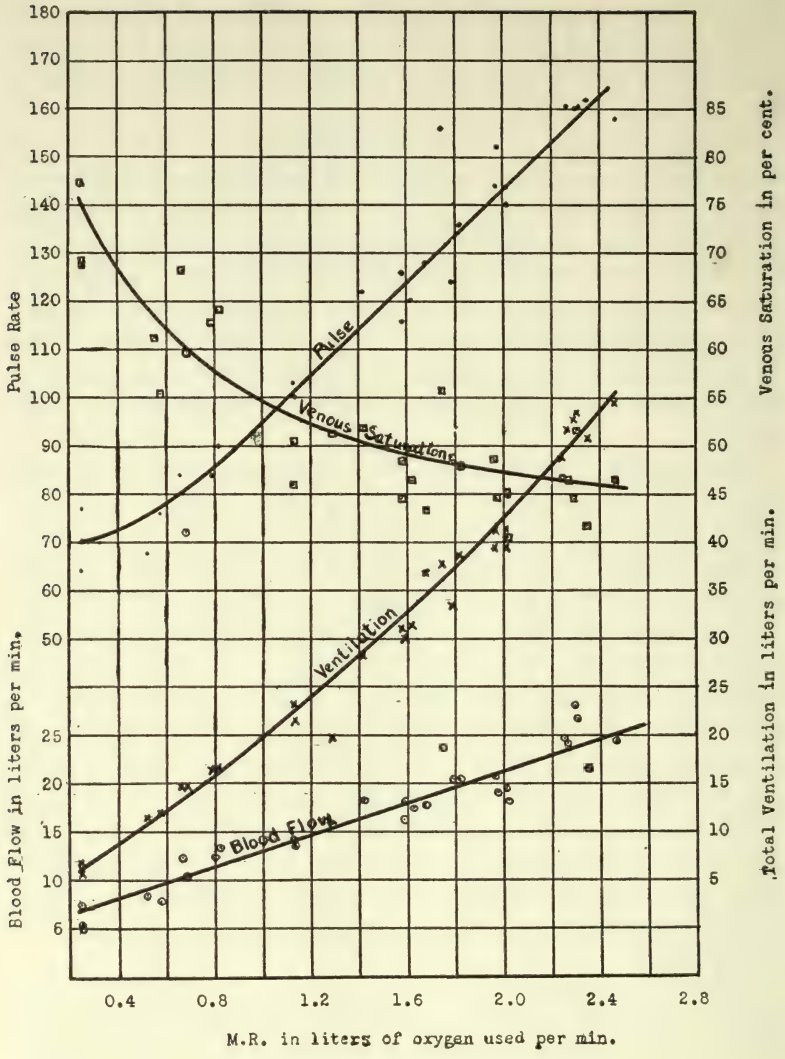

Fig. 217

Physiological Adjustments in Work

ism. The extent of this fluctuation may be judged from inspection of figure 217, on which are recorded all estimates of venous saturation, pulse rate, ventilation, and blood flow as functions of metabolic rate in the case of D.B.D. For this subject measurements are the most numerous. In each experiment on all four subjects work was maintained at a nearly constant level for a period ranging from a few minutes to an hour. All measurements, including numerous analyses of blood and especially estimates of the level of the carbon dioxide dissociation curves and of the lactic acid content, indicate that after a period of two to four minutes a nearly stationary state is reached in De Mar, D.B.D., and A.V.B. In C.V.C. it seemed to be impossible to establish a stationary state for a considerable period at the higher rates of metabolism.

The results of about 90 experiments are summarized in tables 87 and 88. These tables are obtained from smooth curves for all four subjects similar to those of figure 217 for the case of D.B.D. These curves are drawn as follows: The blood flow curve is a straight line fitted to the data by the usual method of least squares. The pulse and ventilation curves are drawn through the experimentally determined points by inspection. The venous saturation curve is calculated from blood flow, metabolic rate, and oxygen capacity, for the variations of which a roughly linear relation to metabolic rate has been found. This is shown on figure 218, in such a manner, however, as somewhat to obscure the uniformity of variation of oxygen capacity with metabolic rate by the simultaneous representation of the random fluctuations of day to day in the composition of blood at rest. The similarity of the slopes of the lines of this figure is an indication of the general uniformity of the effect. The flatness of the curve for A.V.B. may possibly be due to approach to a limiting value.

Blood flow, total ventilation, and pulse rate curves for all four subjects are given on figure 219. Venous saturation curves are shown on figure 220, curves for the stroke

TABLE 87.

Changes during exercise.

A. SUBJECT, D.B.D.

Oxygen used	Pulse rate		Total ventilation		Blood flow		Stroke volume		Venous saturation	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
cc./min.			*l./min.*		*l./min.*		*cc.*		*per cent*	
250	70	100	6.5	100	6.9	100	99	100	75.3	100
500	74	106	10.5	161	8.9	129	120	121	65	87
750	82	117	15.0	230	11.0	160	134	135	58.7	77
1,000	95	135	20.0	307	13.0	188	137	138	54.6	72
1,250	107	153	25.0	384	15.0	217	140	141	51.7	69
1,500	119	170	30.0	461	17.1	248	144	145	49.8	67
1,750	131	187	36.0	553	19.1	277	146	147	48.2	64
2,000	143	204	42.5	653	21.2	307	148	149	47.2	62
2,250	155	221	49.3	757	23.2	336	150	151	46.2	61
2,500	167	240	56.5	870	25.3	367	152	153	45.8	61
					B. SUBJECT, A.V.B.					
250	88	100	5.5	100	5.4	100	61	100	73.7	100
500	93	106	9.9	180	7.3	135	78	128	63.7	86
750	99	115	15.0	272	9.2	170	93	152	58.0	79
1,000	107	121	20.8	378	11.1	206	104	170	54.2	74
1,250	115	130	27.5	500	13.0	241	113	185	51.7	70
1,500	126	143	34.5	627	14.9	276	118	193	49.9	68
1,750	138	157	42.0	763	16.8	311	122	200	48.6	66
2,000	150	170	50.2	912	18.7	346	125	205	47.5	65
2,250	163	185	58.8	1,070	20.7	383	127	208	47.0	64
2,500	177	201	67.5	1,220	22.6	419	128	210	46.3	63

C. SUBJECT, C.V.C.

250	90	100	6.8	100	6.3	100	70	100	75.2	100
500	96	107	12.0	176	7.6	121	79	113	62.0	82
750	102	113	17.5	257	8.9	141	87	124	53.7	71
1,000	111	123	23.2	341	10.1	161	91	130	46.9	62
1,250	122	135	29.2	430	11.4	181	93	133	42.2	56
1,500	134	150	35.3	520	12.7	201	95	136	38.8	52
1,750	146	162	41.6	612	13.9	221	95	136	35.6	47
2,000	160	177	48.0	706	15.2	241	95	136	33.5	44

D. SUBJECT, DE MAR.

250	58	100	8.0	100	8.2	100	141	100	79.8	100
500	64	110	12.9	161	10.0	122	156	110	70.2	88
750	71	123	17.4	217	11.8	144	166	118	63.8	80
1,000	79	136	22.3	279	13.6	166	172	122	59.2	74
1,250	89	153	27.6	345	15.5	189	174	123	56.2	70
1,500	98	170	33.3	415	17.3	211	177	126	53.7	67
1,750	108	186	39.0	487	19.2	234	177	126	52.0	65
2,000	118	203	44.8	560	21.0	256	177	126	50.5	63
2,250	129	222	50.8	635	22.8	278	177	126	49.3	62

TABLE 88.

Summary of respiratory data.

A. SUBJECT, D.B.D.

Oxygen used *cc./min.*	Respirations per min.	Total ventilation *l./min.*	Tidal air *cc./min.*	CO_2 eliminated *per cent*	O_2 used *per cent*	R.Q.	Alveolar CO_2 *mm.*
250	14	6.5	460	3.25	3.90	.83	36.5
500	15	10.5	700	3.95	4.60	.86	38.0
750	16	15.0	940	4.28	4.84	.88	39.3
1,000	17	20.0	1,180	4.50	4.95	.91	40.5
1,250	18	25.0	1,390	4.69	5.00	.94	41.0
1,500	19	30.0	1,580	4.80	5.00	.96	41.5
1,750	20.5	36.0	1,760	4.80	4.93	.97	42.0
2,000	22	42.5	1,930	4.76	4.80	.99	42.0
2,250	23.5	49.3	2,100	4.60	4.59	1.00	42.0
2,500	25	56.5	2,260	4.30	4.30	1.00	42.0

B. SUBJECT, A.V.B.

250	7	5.5	790	3.60	4.30	.82	36.0
500	11.5	9.9	860	3.83	4.53	.85	36.7
750	15	15.0	1,000	3.98	4.60	.87	37.5
1,000	18	20.8	1,160	4.10	4.61	.89	38.0
1,250	19.5	27.5	1,410	4.17	4.58	.91	38.2
1,500	21	34.5	1,640	4.19	4.50	.93	38.3
1,750	23	42.0	1,830	4.15	4.39	.95	38.4
2,000	25	50.2	2,000	4.10	4.23	.97	38.5
2,250	27	58.8	2,180	4.00	4.06	.99	38.5
2,500	28.5	67.5	2,370	3.89	3.85	1.00	38.5

C. SUBJECT, C.V.C.

250	16	6.8	420	3.10	3.95	.80	40.0
500	21.5	12.0	560	3.65	4.14	.88	40.5
750	25.5	17.5	690	3.92	4.28	.92	40.7
1,000	28.5	23.2	810	4.24	4.39	.99	41.0
1,250	30.5	29.2	960	4.45	4.45	1.00	41.0
1,500	32.0	35.3	1,100	4.50	4.45	1.01	41.0
1,750	33	41.6	1,260	4.45	4.39	1.01	41.0
2,000	34	48.0	1,410	4.32	4.25	1.02	41.0

D. SUBJECT, DE MAR.

250	16	8	500	2.70	3.40	.79	39.0
500	18.5	12.9	700	3.12	3.70	.84	40.0
750	20.5	17.4	870	3.50	3.98	.88	40.5
1,000	23.0	22.3	970	3.80	4.22	.90	41.0
1,250	25.5	27.6	1,080	4.04	4.42	.91	41.0
1,500	28	33.3	1,190	4.19	4.58	.91	41.2
1,750	31	39.0	1,260	4.28	4.66	.92	41.5
2,000	34	44.8	1,320	4.30	4.54	.95	41.5
2,250	36.5	50.8	1,410	4.29	4.38	.98	41.5

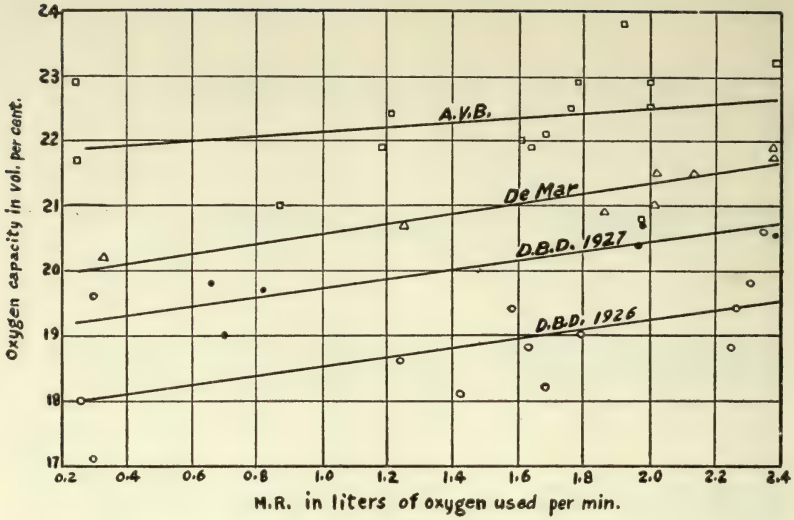

Fig. 218

Oxygen Capacity and Metabolic Rate

volume of the heart on figure 221, and curves for tidal air on figure 222. It is evident that, according to these studies, the output of the heart throughout the interval $2400 > MR > 250$ is approximately a linear function of metabolic rate. The average for the four subjects, expressing both blood flow and oxygen consumption in liters per minute, is represented with fair accuracy by the equation:

$$BF = 7MR + 5,$$

and this may be taken as an estimate of the conditions in most normal men.

The pulse rate is more variable from case to case, and, in that of De Mar, the slow rate of the athlete, noted by Y. Henderson[150b] and others, is conspicuous. On the whole these pulse rates give an excellent indication of the physical efficiency of the four subjects.

[150b] Henderson, Haggard, and Dolley, *American Journal of Physiology,* LXXXII, 512 (1927).

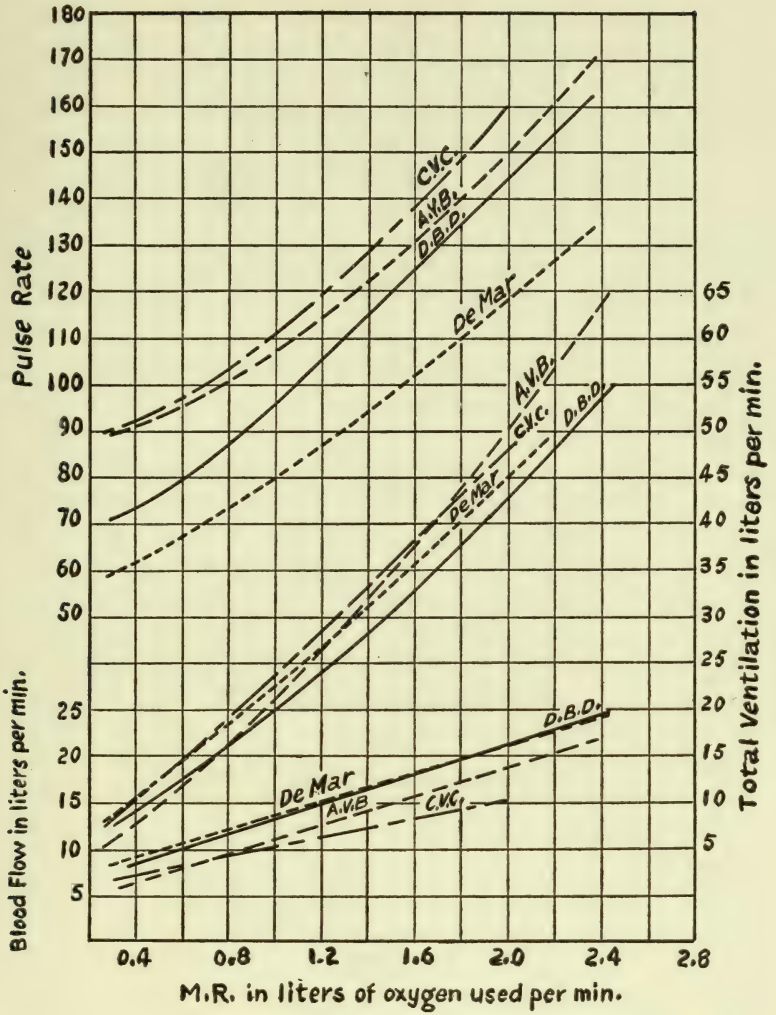

Fig. 219

Physiological Adjustments in Work: Comparative Studies

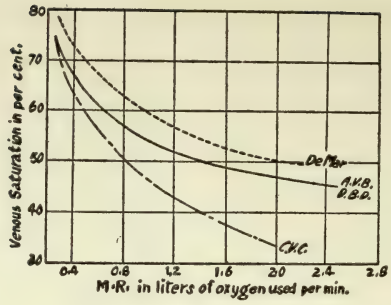

Fig. 220

Venous Saturation and Metabolic Rate

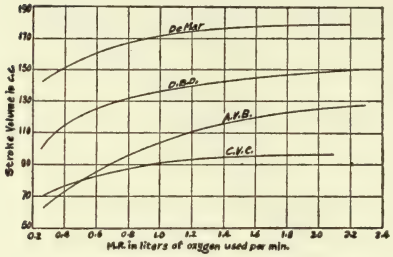

Fig. 221

Stroke Volume and Metabolic Rate

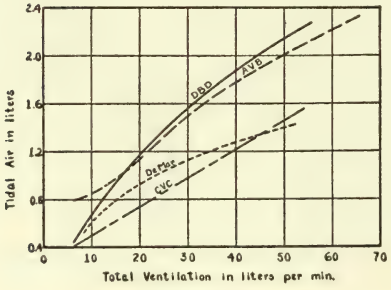

Fig. 222

Tidal Air and Total Ventilation

Taking account of the similarity of the blood flow in all four cases, it is evident that the stroke volume of the heart must be especially dependent upon the pulse rate, and therefore variable. It will be seen that for C.V.C. the stroke volume is but 70 cc. at rest and rises to nearly 95 cc., while for De Mar corresponding values are 141 cc. and 177 cc. respectively. These measurements are consistent with some of the published measurements of the same quantity and inconsistent with others, since physiologists have been far from agreeing in this question. However, in view of the nature of the experiments now in question, and particularly of the consistency of all the results, I think that they may be regarded as not very inaccurate. It will be borne in mind that in two persons having blood of the same composition, especially the same oxygen capacity and hydrogen ion concentration, the transport of the same quantity of oxygen during moderate muscular activity may be expected to require the movement of about the same volume of blood. If this be so, the pulse rate, about which there can be no question, must be roughly inversely proportional to stroke volume for corresponding states in different normal subjects.

The total ventilation of the lungs is roughly the same in all four subjects, though in A.V.B. the condition seems to be somewhat anomalous. This subject is peculiarly liable to mountain sickness, the diffusion coefficient of his lungs as measured by Krogh's method is low, and, as the tables show, his respiratory rate at rest is very slow. This last fact is perhaps related to the peculiar s-shape of the curve of his tidal air. On the whole, respiratory rate and tidal air vary widely and in a manner that is hard to interpret. There is to be noted, however, one striking uniformity in the respiratory variation of all four subjects. With increasing metabolic rate the relative quantities of oxygen removed from inspired air and of carbon dioxide added to it both increase, pass through a maximum, and then fall. These maxima occur in the different subjects at different metabolic rates as follows:

	Maximum of O_2 removed from inspired air Metabolic rate	Maximum of CO_2 added to inspired air Metabolic rate
De Mar	1750	2000
D.B.D.	1350	1650
A.V.B.	1000	1500
C.V.C.	1350	1500

The respiratory quotients pass through very similar changes in all four subjects, but increase most rapidly in C.V.C., least rapidly in De Mar, with increase in metabolic rate. Unlike the other variables above considered, respiratory quotients manifest certain characteristic changes as the experiment is prolonged. For a period slightly less than a half hour the values observed are those recorded in the table, thereafter a gradual fall occurs. This fact, mentioned in an earlier chapter, has now been fully established by several careful experiments and the results of one of them are given in figure 223. The change is no doubt associated with depletion of the glycogen supplies of the body.

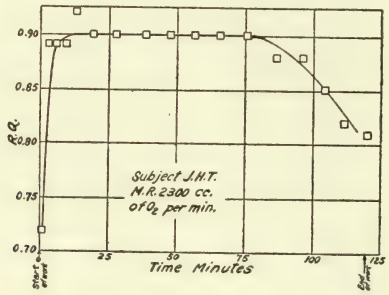

Fig. 223

Respiratory Quotient as a Function of Time

Extensive and remarkably consistent observations of this kind have led Bock and Dill to the conclusion, or speaking more precisely, to the working hypothesis that the changes of the respiratory quotient in work, after

making proper allowance for the liberation of carbon dioxide by the action of acid, are due to two processes: (1) Increased oxidation of carbohydrate in the chemical activities involved in the mechanism of muscular contraction and (2) increased formation of carbohydrates from other substances, or increase of some other process by which other substances are utilized. They assume, further, that the first process greatly outruns the second in earlier stages of muscular work, so that the second may be entirely masked, but that the velocity of the second process probably increases as the stores of glycogen are depleted. They assume, finally, that the rate of the second process is more nearly equal to that of the first process in trained athletes and that this explains their observation that the magnitude of the respiratory quotient is a good index of performance. This hypothesis, which is in agreement with the views of some other investigators, seems to fit all the facts and, in the first of the alternative forms, it has the advantage of avoiding the assumption that so intricate a process as muscular contraction can take place by means of the oxidation of substances of widely different constitution. In view of the history of this question, it is well, nevertheless, still to distinguish carefully between fact and hypothesis.

From the standpoint adopted in this book, variations in the oxygen content of venous blood are especially significant. Inspection of the figures and tables will show that in all four subjects, as metabolic rate increases, oxygen content of venous blood falls. This fall is at first rapid, later slow, and finally in De Mar, D.B.D., A.V.B., it seems to approach the limits of 50 per cent, 46 per cent, and 46 per cent respectively. In C.V.C. there is no definite indication of a limit, probably because at high rates there is a steadily increasing acidosis and no stationary state. The lowest value reached in this subject is 33 per cent.

These variations of the composition of mixed venous blood at a metabolic rate of two liters of oxygen per minute are associated with varying degrees of acidosis in the

four subjects. From analyses of samples of blood withdrawn during the course of the experiments, the extent of the acidosis may be calculated. The facts concerning the values of pH_s for the arterial blood of the four subjects are summarized in table 89. These changes of the quantity pH_s may perhaps be regarded as the best index of the performance of the four subjects.

TABLE 89.

	MR 250	MR 2,000	
Subject	pH_s	pH_s	ΔpH_s
De Mar	7.42	7.40	0.02
D.B.D.	7.44	7.38	0.06
A.V.B.	7.45	7.36	0.09
C.V.C.	7.42	7.27	0.15

Taking account of the variations in the alkalinity of the blood, and of the accompanying changes in affinity for oxygen, it is possible to calculate the value of the pressure of oxygen in the mixed venous blood for the condition of work at MR 2000 as follows: De Mar, 24.5 mm.; D.B.D., 24.0 mm.; A.V.B., 24.0 mm.; C.V.C., 23.0 mm. Evidently, during work of this intensity, the blood leaving the capillary of an active muscle contains oxygen at a nearly constant partial pressure of about 20 mm.

It will be remembered that the difference in partial pressure of oxygen between alveolar air and arterial blood is also about 20 mm. In spite of the difference in conditions we may perhaps draw the conclusion that the pressure of oxygen in the active muscle is nearly 0 and that the oxidation processes of muscular activity take place at a concentration of free oxygen of the order of not more than $10^{-5}N - 10^{-6}N$. This conclusion, which seems to rest upon a secure foundation, should be of value in the study of the physico-chemical processes in which oxygen takes part. It is also serviceable as a means of defining the nature of the diffusion process.

Accordingly, we may next consider the mean head of

oxygen pressure, $\Delta p'$, in the muscle capillaries. This varies in the manner described in Chapter VIII with the values of pH_s. It also varies, as also explained, with the coefficient of utilization of oxygen. Finally, it varies in a manner not yet discussed with the respiratory quotient. The nature of this variation may be readily understood from a study of any blood nomogram. On such a nomogram the scales of pH_s and pH_c form with the arterial and venous blood lines approximately similar triangles. As long as the respiratory quotient remains constant, the respiratory variations in values of pH_c are therefore proportional to the accompanying variations in pH_s. But, as the value of the respiratory quotient increases, the size of the smaller triangle approaches that of the larger triangle and the variations of pH_c become larger relatively to the variations of pH_s. For a respiratory quotient of 1.00, variations in pH_c accompanying a given variation in pH_s are more than double the variations for a respiratory quotient of 0.80. Taking account of all of these factors the values of $\Delta p'$, or mean head of oxygen pressure, for the capillaries of active muscles in the four subjects of these experiments at MR 2000 are as follows: De Mar, 45.1 mm.; D.B.D., 45.0 mm.; A.V.B., 45.7 mm.; C.V.C., 45.7 mm. It is hardly possible that this precise agreement in the results of intricate calculations which involve nearly all the theoretical considerations discussed in earlier chapters is not partly fortuitous. Nevertheless there can be little room for doubt that in the capillaries of the active muscle of a normal man this value is approximately constant. Such a result seems to give very strong support to the data upon which it is based and leads me to believe that the studies of Bock, Dill, and their associates can hardly be affected with systematic errors of more than moderate size or with random errors that are more than small.

At a metabolic rate of 2000 a value of $\Delta p'$ of 44 (a value obtained by making a small allowance for blood flowing through inactive parts of the body) implies a diffusing capacity of about 45, or a diffusing capacity per liter of

oxygen per minute of 22.5. In view of the identical results for all four subjects this may be regarded as the normal value for active muscles, and it may be assumed provisionally that this value depends upon a fairly exact regulation of the velocity of the blood stream in the capillaries of the muscle, which is probably of the same order as the velocity in the capillaries of the lung.

If the above discussion, necessarily hypothetical in certain respects, has not led us into serious error, it seems probable that, aside from mild acidosis, the properties of blood in the capillaries of the active muscle and also the character of its motion are roughly constant. I have in the past suggested this interpretation of the facts as a working hypothesis[151] and now venture to repeat the suggestion. It must be understood, however, that proof of the theory, if indeed it can be established, will involve much further experimental work. For the present too many of our variables remain inaccessible to measurement.

Not the least important aspect of the work of Bock and Dill is the precise and intelligible definition of differences between the four subjects of their experiments. With the exception of a few features such as the slow breathing of A.V.B. at rest and the rapid, shallow breathing of De Mar at work, mere personal idiosyncrasies are inconspicuous and all the physiological functions that have been studied vary in a regular manner from case to case, the order of variation being invariably De Mar, D.B.D., A.V.B., C.V.C. This is clearly the order of their ability to perform muscular work, and with the experiments in view it would not be difficult to assign to their abilities numerical coefficients in each case. For the present, however, we have no immediate use for such coefficients.

All the differences between the four subjects may, no doubt, be associated with the differences in the performance of work. For example, there is no reason to doubt the great importance of the differences in stroke volume of the heart between De Mar and C.V.C. Nevertheless,

[151] Dale, Drummond, Hill, and Henderson, *Lectures on Certain Aspects of Biochemistry,* London, 1926.

under the conditions of these experiments, there are no signs of failure of the heart to meet the needs of the organism, and the indications of such failure seem to be exclusively chemical. In C.V.C., as compared with De Mar, there is striking inability to complete the processes of oxidation, lactic acid is accumulating, and the chemical mechanism of the muscle is becoming deranged. Secondly, the values of the respiratory quotient (at MR 2000, for C.V.C., 1.02; for De Mar, 0.98; at MR 1000, for C.V.C., 0.99; for De Mar, 0.90) suggest that in C.V.C. there is also a slow rate of production of carbohydrate or of other materials for use in the muscle. These facts have led Bock and Dill to the hypothesis that in such cases the capacity to perform work depends upon the velocity of the chemical reactions involved in these processes, and it is in this direction that they look for a clearer interpretation of the phenomena of training and of fatigue. Here their studies come in contact with those of Hill, of Meyerhof, and of Fiske.

Differences between individuals, whether constitutional or acquired, are relatively small among normal men and, if we would find the widest range of variation in the physiological activities discussed in this chapter, we must look to the clinic, as we did in seeking extreme variations in the properties of the blood. As yet, however, our studies have not been extended in this direction and on this subject little remains to be considered beyond what has been considered already in Chapter X. Nevertheless, with the changes of the normal organism during muscular activity clearly defined, it becomes less difficult to define the limitations imposed by pathological states.

Let us consider the obvious relation that metabolic rate is equal to blood flow multiplied by the quantity of oxygen per liter of blood absorbed in the lung and given off in the tissue:

$$MR = BF \times \Delta O_2 \text{ cc.}$$

As the oxygen capacity of the blood varies, so, other things being equal, does the quantity of oxygen (ΔO_2 cc.

in Murray and Morgan's notation) that can be transported. Therefore, within certain ranges, the maximum attainable metabolic rate must be more or less proportional to the oxygen capacity of the blood. Hence the disability of anemia. There are two important qualifications of this statement. First, as oxygen capacity increases above the normal, a point must be reached where blood flow is slowed by increasing the viscosity of the blood. This is perhaps one of the limiting factors in the case of congenital defect of the heart presented in Chapter X. Hence we must say that the above equation holds even approximately only at and below the normal ranges of oxygen capacity of blood. Secondly, given adequate ventilation of the lung, which, as in nephritis and diabetic coma, may involve extreme respiratory activity, the quantity of oxygen carried per liter of blood increases with the acidity of the blood, as in the normal man, C.V.C. And also, as already explained, there may be adaptive changes of unknown origin in the affinity of hemoglobin for oxygen.

Perhaps, in view of these facts, we may venture a hypothesis that a low grade of transitory acidosis may be both effective in increasing the amount of work that can be done, and otherwise harmless. But, if we consider the case of nephritis of Chapter X, it can hardly be doubted that the acidosis is doing far more harm than good. In short, the variation of ΔO_2 cc. with constant oxygen capacity of blood is possible only within certain limits, while useful variation of this quantity is possible only within fairly narrow limits.

Bearing in mind the corrections that must be applied for acidosis and for otherwise varying affinity of hemoglobin for oxygen, it is evident that the blood flow of the resting state must increase as oxygen capacity of blood falls. Thus an unavoidable increase of the work of the heart is necessitated. For example, when the oxygen capacity of the blood is reduced to one-fourth its normal value the rates of blood flow must be at least nearly trebled. In simple anemia this is perhaps unimportant,

but in severe nephritis it appears to be a pathological feature of considerable moment. With increasing metabolic rate this disability becomes more and more pronounced and, in view of the conclusions reached in the present chapter, it is not difficult to see that the case of nephritis described in Chapter X presents a condition in which considerable increase of the metabolic rate beyond that of the resting state is quite impossible. Further analysis of these questions must await the results of further experimental observations.

As for the other species of animals whose bloods are described in Chapter XI, it must suffice to point out that similar treatment of the problems of their circulatory and respiratory functions is possible. It may be added that, in the case of the horse and of mammals in general, similarity in composition of the physico-chemical system blood implies a similarity in the properties of the circulation and of the respiration, but, at the same time, we must remember that differences in size, involving the application of the principle of similitude, greatly modify such processes even when the chemical composition remains unaffected.

In the turtle differences in the blood are associated with differences in the circulatory apparatus. As a result of our observations it seems probable that for this form the flow of blood is so adjusted that a portion of the blood which has passed through the lung is mixed with venous blood in the heart and passes again through the lung, while the blood which enters the greater circulation from the heart may perhaps be highly oxygenated. Further experiments will be necessary to settle this question, which presents difficulties for those not well acquainted with the anatomical relations involved. Our observations at least show that the application of modern methods of blood analysis should, in the long run, contribute to the progress of the comparative physiology of the circulation.

We have now taken into consideration the relations between the respiratory cycle of blood, the circulation, the respiration and the function of the muscular system.

These are all quantitatively large activities and it is partly because they are large that the mutual relations between them may be readily defined and theoretically interpreted. It is also for this reason that we have chosen them for investigation. But there are other large physical and chemical activities of the organism which we have left out of account. Among these is the formation of urine. I have in the past discussed the theory of the regulation of the acid-base equilibrium of the body by means of variation of renal action[152] and the phenomena were extensively studied by Palmer and myself.[153] Recently this question has received much attention and in particular the researches of Gamble[154] have greatly extended our knowledge of inorganic and acid-base metabolism. It is not difficult to see that the activities here in question may be correlated by means of suitable experiments with the other physiological activities discussed in this book. Thus, and by similar means, it will be possible to broaden the scope of our physiological synthesis.

But, before the subject of the present chapter can be treated on the same scale and with the same precision that are now possible in the exposition of the physico-chemical properties of blood, there will be required not only a broadening of the scope of experimentation to include the activity of the kidney and other functions, but also a far more detailed investigation of the mutual relations of circulation, respiration, and metabolism in pathological conditions and in other species.

[152] L. J. Henderson, *Journal of Biological Chemistry,* IX, 403 (1911).

[153] Palmer and Henderson, *Archives of Internal Medicine,* XII, 153 (1913); Henderson and Palmer, *Journal of Biological Chemistry,* XIII, 393 (1913); Henderson and Palmer, *Journal of Biological Chemistry,* XIV, 81 (1913).

[154] J. L. Gamble, S. G. Ross, and F. F. Tisdall, *Journal of Biological Chemistry,* LVII, 633 (1923); J. L. Gamble, K. D. Blackfan, and B. Hamilton, *Journal of Clinical Investigation,* I, 359 (1928); J. L. Gamble and S. G. Ross, *Journal of Clinical Investigation,* I, 403 (1925); J. L. Gamble and B. Hamilton, *Bulletin of Johns Hopkins Hospital,* XLI, 389 (1927).

CHAPTER XIII

CONCLUSION

THE inquiry brought to an end in the last chapter has proceeded by stages as follows: In the beginning we made an inventory of those aspects of general physiology to which the respiratory activity of blood is unmistakably related. Next, we defined the chemical composition of blood in such a way that a quantitative though roughly approximate study of the physico-chemical system should be possible. There followed a consideration of the partial activities that have been heretofore recognized in the system and a nomographical synthesis of these parts into a description of the conditions of equilibrium in a single specimen of blood. The nomogram thus obtained was then utilized to define and to analyze, perhaps in excessive detail, the respiratory cycle of the blood. Thereafter it became possible to take account of the relations between the properties of the blood and its cycle, on the one hand, and those of the respiration and circulation, on the other hand. The rest of the study is comparative; it comprises, first, the quantitative description of the physico-chemical system in rest and work, in health and disease, and from species to species; secondly, a corresponding description of the respiratory cycle of blood; and thirdly, a description of the mutual dependence of the properties of blood and of the circulation, respiration, and metabolism.

The important features of this treatment of the problem are three: It is approximate, quantitative, and synthetic. First, it is approximate. This depends not only upon the inaccuracies of experimental measurement, but also upon deliberate neglect of variables that are known

to be involved in the phenomena, such as phosphates as components of the physico-chemical system, the exchange of other components beside oxygen and carbon dioxide in the capillaries as a part of the respiratory cycle, and the activity of the kidneys associated with the activities of the heart, the lungs, and the muscles. In physiology such simplifications are indispensable, except in rare instances when, as is generally the case in physics and chemistry, it is possible to eliminate the variables experimentally. The resulting inaccuracy is a small price to pay in order to achieve the simplification. This is, in fact, the necessary condition for quantitative treatment of the phenomenon.

The history of this branch of physiology illustrates the point. Thus equilibrium between carbonic acid and blood was first described by means of a single carbon dioxide dissociation curve, and the facts concerning the heterogeneous equilibrium between cells and plasma, though well known, were neglected. Later it became possible to add a consideration of the acid-base equilibrium and of hydrogen ion concentration. This was followed by discovery of the effect of oxygenation, and the single curve became a family of curves. At length the construction of a nomogram made possible the simultaneous consideration of all the first order physico-chemical variables, including those of the previously neglected heterogeneous equilibrium. At this stage the variable, time, made its appearance and the main features of the carbonic acid cycle, previously only guessed at, were defined. Moreover, since the nomogram existed, these features were defined as parts of the cycle of the complete physico-chemical system. Finally, the changes of the carbonic acid equilibrium of the blood which accompany fatigue or arise in diabetic coma, nephritis, anemia, and other conditions, and which had never been clearly related to many of the other factors of the physiology of blood, were added to the description.

Thus a synthesis was gradually attained, for the third feature of the present treatment is the result of the sec-

ond, because a description of a system in which there occur many variables in a state of mutual dependence becomes possible, but becomes possible only with the help of mathematics. Otherwise it is not feasible even to begin to think about the system as a whole, while the study of the parts is, in general, both difficult and misleading. A clear explanation of this important fact may be found in the following words of Pareto:[155] ". . . one of the numerous cases where mathematical language makes possible precision and rigor which cannot be attained by the use of ordinary language.

"Let $x, y, \ldots s, u, v, \ldots$ be indices of the magnitudes of A, B, C, . . . ; the relations between A, B, C, . . . are given by certain equations:

$$(1) \quad \phi_1(x, y, \ldots) = 0, \quad \phi_2(x, y, \ldots) = 0, \ldots$$

All these quantities $x, y, \ldots$ may be functions of the time, t, which may also appear explicitly in the system of equations (1). This system, if we suppose time variable, represents the relations of A, B, C, . . ., and the evolution of these relations. It is only knowledge, even very vague and very imperfect knowledge, of the system (1) of equations which enables us to have any knowledge whatever of the relations and of their evolution. Most authors do not perceive this fact, and ignore the very existence of the system of equations, but that does not prevent their reasonings from taking this system as a premise, without their knowing it.

"If one supposes the equations of the system (1) to be in number equal to the number of unknowns the latter are [in general] all determined. If one supposes that the equations are less in number, which amounts to suppressing by hypothesis certain conditions that really exist, it is possible to take as independent variables $s, u, v, \ldots$ in number equal to the number of equations suppressed and to suppose that $x, y, \ldots$ are functions of these inde-

[155] V. Pareto, *Traité de Sociologie Générale,* II, 1288-1290, note, Lausanne and Paris, 1919.

pendent variables. If we differentiate the equations (1) with respect to the independent variables, we shall have the system

$$(2)\quad \begin{cases} \dfrac{\partial \phi_i}{\partial x}\,dx + \dfrac{\partial \phi_i}{\partial y}\,dy = 0 \\ i = 1, 2, \ldots \end{cases}$$

The total differentials dx, dy, . . . represent *virtual movements* which take place if one supposes that the independent variables s, u, v, . . . change into $s + ds$, $u + du$, . . . These virtual movements are determined by the equations (2).

"From the mathematical point of view the systems (1) and (2), or those into which they may be supposed transformed, are equivalent. One passes from the first to the second by differentiation, from the second to the first by integration. Often the second system is easier than the first to establish directly.

"Whoever ignores everything of one or the other of these two systems ignores also the relations that A, B, C, . . . can assume. Whoever knows something of these relations knows by that very fact something of the systems (1) and (2). . . .

"If there is but a single independent variable, s, this very generally is named the *cause* of the *effects*, x, y, . . ., and the increment, ds, is said to be the *cause* of the virtual movements, dx, dy, . . . When one considers only the relations of cause and effect one operates, from the mathematical point of view, by reducing systems (1) and (2) to the following or to other equivalent systems:

$$(3)\quad \phi_1(x, s) = 0, \qquad \phi_2(y, s) = 0, \ldots$$

$$(4)\quad \frac{\partial \phi_1}{\partial s}\,ds + \frac{\partial \phi_1}{\partial x}\,dx = 0, \qquad \frac{\partial \phi_2}{\partial s}\,ds + \frac{\partial \phi_2}{\partial y}\,dy = 0, \ldots$$

These two systems of equations are easier to treat than systems (1) and (2), either in ordinary language or even by means of mathematics. It is, therefore, advantageous

whenever possible to substitute them for systems (1) and (2). Cases exist where one reaches in this manner at least a grossly approximate solution of the problem that one has undertaken to solve. In other instances this is impossible, and then the substitution of systems (3) and (4) for systems (1) and (2) cannot take place, for it would lead to results having nothing to do with reality.

"From the mathematical point of view it is well known that the integration of system (2) does not simply reproduce system (1), but yields very much more extensive solutions, among which system (1) is comprised. In order to determine system (1) completely it is, therefore, necessary to add other considerations. In like manner the integration of system (4) not only reproduces system (3), but also introduces arbitrary constants which must be determined from other considerations. This is a very general phenomenon in the application of mathematics to concrete facts. Even in the elements of algebra, when the solution of a problem is given by an equation of the second degree, there is often one root which suits the problem while the other does not, and must be rejected."

* * * * *

As a result of the absence of metabolic activity in ordinary human blood and of the gaseous nature of oxygen and carbon dioxide, it has been possible to push the researches described in these chapters much farther than is ordinarily possible in the physiological laboratory. It is for this reason that at present there exists no other description of physiological integration on so large a scale. But the present synthesis is still very imperfect. Let us seek to make this clear by a single illustration. Throughout these chapters we have adopted the approximation that all mammalian blood may be described (in respect of those physiological functions to which we have limited our inquiry) as a system consisting of eight components. Results have justified this as a useful and sufficiently accurate approximation. This being the case, it is evident

that the *general* problem of comparative physiology of blood is to be solved not merely by the construction of a large number of nomograms in which there are two independent variables, oxygen and carbon dioxide, but also by treatment of the single system of eight components and eight independent variables. From this goal we seem to be still remote, though it is possible that carbon dioxide dissociation curves may yield to a determined attack sooner than some will expect. In this connection, it should be also remembered that the lack of a direct method for the measurement of blood flow is a serious disadvantage and ignorance of oxygen pressure in the tissues a grave disability. For these reasons, many of the discussions of the preceding chapters suffer from a lack of precise information. But there is perhaps no limit to what may be discovered by experiment and, though the questions here involved are often trivial, so long as there are no other means of studying physiological synthesis broadly, even trivial problems in this field will assume special importance for those who are interested in general physiology, and experimentation will continually yield new results.

The synthesis which we have reached is not only imperfect in these respects; it is also very incomplete. But, in truth, a complete physiological synthesis is quite inconceivable and anything that might be regarded as an acceptable approximation to a complete synthesis hardly less so. Nevertheless, I think it is evident that progress has been made toward understanding the mutual dependence of the quantitatively large physiological processes in normal man at rest and at work. If for disease the description is as yet even less complete, this is due to no insuperable difficulties. Also there can be no reason to believe that there is any limit to progress, through the introduction of more and more variables in the analysis of the facts, through increase in accuracy of experimentation, and through improvements in the mathematical methods, which are at present, I fear, often primitive and inadequate.

Although pathology has been one of the least of our concerns in these chapters, I think that the pathological results of our inquiry are not its least significant results. The science of pathology is notoriously backward and non-rational. During the epoch of pathological anatomy, from the taxonomical period of Morgagni and his successors to the cellular period of Virchow, this science no doubt held its own with the other medical sciences, but, upon the appearance of the bacteriological period in the history of pathology, an intellectual decline, as I think it may be fairly regarded, set in. In accordance with the simple views of Pasteur, the specific virus came to be regarded as the *cause* of each infectious disease and this view could hardly be questioned during the time of rapid progress in discovery of microörganisms. The hypothesis is roughly correct, but it has obscured the organic character of disease and the mutual dependence of the many variables which must be taken into account, if the state of the patient is to be understood. Yet the facts can never be quite overlooked by the experienced physician, for whom the complex reality is always present. This is one of the reasons for the conflict of the laboratory and the clinic.

Among the results of our present inquiry is the demonstration that an understanding of the mutual dependence of the physiological facts is impossible without a mathematical analysis based upon measurement. As we have seen, even a rough and extremely incomplete synthesis is otherwise beyond our reach, for without mathematics we can hardly begin to think about mutual relations between more than two or three variables. This has long been known to the mathematicians, for economics it was fully demonstrated by the work of Walras and explained, as above set forth by Pareto, but even yet the fact is hardly appreciated by physiologists. The difficulty may be illustrated, for anyone who is acquainted with the physiology of respiration, by asking the question: What is the course of events leading to death in a man imprisoned in a

sunken submarine? From the standpoint of the physicist no simpler modification of the conditions is conceivable, yet a fairly satisfactory answer to the question is impossible. Such an answer is, however, not now unattainable and further studies of the mutual dependence of the properties of the air, of the blood, of the circulation, and of the respiration, similar to those which we have considered, will yield an answer.

In disease, conditions are perhaps no more complex, since the normal organism is as complex as the diseased organism. Nevertheless, a larger number of unknown modifications are ordinarily involved and the problem is correspondingly difficult. Yet it is unmistakable that the very imperfect descriptions of the state of the organism in nephritis, in diabetic coma, and in other pathological conditions which we have studied do afford a different kind of understanding of the patient from that given by the usual studies of acidosis, of renal insufficiency, and of similar abstractions of pathology.

Here the important consideration is not the advance that has been made, which is small; it is the character of this advance. We have found that by means of measurements and mathematical analysis something can be learned of the state of the whole diseased organism. Now if something can be learned, then much can be learned. Therefore, the time must come when the science of pathological physiology, conceived as the study of the mutual dependence between many variables, will afford descriptions of disease that partly meet the long-felt needs of physicians. It is hard to believe that such descriptions can ever be adequate, for they must remain roughly approximate. And no doubt intuition, as we vaguely say, must forever serve the physician in lieu of fact, as it served Hippocrates. But, at least, science and intuition will tend to become congruent, and intuition more rational.

The French School has lately devoted much labor to the study of regional geography, according to a method which may serve to fix our ideas. It is recognized that a state of

mutual dependence exists between such factors as soil, relief and climate, means of communication, types of habitation and distribution of population, commerce and industry, etc. Accordingly, these features of the problem are studied and so far as possible are treated quantitatively. Next, the historical, racial, and political factors are examined. Finally, since such methods can at best yield but a rough approximation to a description of the complex reality, the work takes on a literary form, the imagination aiding in the process of integration, and thus a completion is reached.[156] The imagination so employed is a dangerous guide, in medicine as in geography, and yet it may be hoped that medical monographs will sometimes in the future follow this plan. Thus the description of a disease, founded upon mathematical physiology and presenting a crude but approximate description of the whole organism, enlarged by the insight and experience of the skilful physician, may yet become a bridge between the laboratory and the clinic.

The disease, however, is an abstraction or approximation, and the sick man is the concrete fact. So medical science comes finally to the individual, and it is individuals that we have studied in this book.

The logical confusion between the class and the individual, a source of endless strife in the history of philosophy, is another reason for the conflict between laboratory and clinic. But, here again, there is some ground for hope that mathematical physiology may lessen the conflict. We have noted in these chapters measurable differences between normal men, between men suffering from the same disease, and of course such differences are well known to exist. It is not too much to hope that, by study of the mutual dependence between the variables, differences in constitution, temperament, susceptibility to disease, etc., may be defined. Here what is wanted is a scientific analysis of the individual, founded not merely upon anthropological

[156] See, for example, R. Blanchard, *La Flandre,* Dunkerque, 1906.

measurements, or psychological tests, or the study of the internal secretions, but also upon the quantitative study of the quantitatively great physiological activities, and above all upon the analysis of the mutual dependence of all these factors. In this manner, it seems probable that an exact science of anthropology or human biology may be slowly built up.

The persistent study of the mutual dependence between the physiological variables leads very slowly to a better understanding of concrete reality or, in other words, to a description of the whole organism. In particular, we reach a conception of the organism as an immensely complex system in equilibrium (which must not, however, be confused with thermodynamic equilibrium, in the strict sense) a view that Pareto[157] has clearly worked out for society: ". . . accidental changes of an element which arises, acts for a short time on a system, producing in it a slight deviation from the state of equilibrium, then disappears. For example, short wars for a rich country, epidemics, floods, earthquakes, and similar calamities, etc. Statisticians had already remarked that these events interrupt, for a brief period only, the course of economic and social life; but many investigators, lacking the notion of equilibrium, set to work to discover imaginary causes. This is what happened to Stuart Mill, seeking why a country, disturbed for a brief period by war, quickly returns to its previous state. On the contrary, others like Levasseur invoked a mysterious 'law of compensation.' The equilibrium of a social system is similar to that of a living organism. Now, from the earliest times, the reestablishment of equilibrium, accidentally and slightly modified in the living organism, has been observed. As usual, men have wished to give a metaphysical color to this phenomenon and have invoked the *vis medicatrix naturae.*" This statement, which has many implications, suggests in a very general form a dynamical conception

[157] V. Pareto, *Traité de Sociologie Générale,* II, 1309, note, Lausanne and Paris, 1919.

of survival, but one that is probably too abstract for the present purposes of the physiologist.

Yet all the comparative studies of these chapters illustrate the principle and, by changing the point of view, physiological considerations may be made clearer. We may, for instance, turn our attention to the properties of the substances and of the structures that are involved in the disturbance of the physiological equilibrium, and seek a measure of their share in the restoration of this state. Here we shall find quantitative relations that may be defined as efficient. Again we may study the degree of relatedness of the parts of the organism. This we shall find to be very great, and this property also is very effective in the preservation of the stationary state of the organism. We may then say, as has been pointed out in the first chapter, that the organism is adapted and adaptable. The law of adaptation in organisms is for the physiologist hardly less general or less convenient than the second law of thermodynamics for the physicist. I shall not now endeavor to go beyond what has been said above on this point.

The adaptive character of the phenomena has been expounded at length in the preceding chapters. It has been noted in the physico-chemical properties of the components of the system, *e.g.,* in those of hemoglobin, in the changes of the composition and state of equilibrium of the system, *e.g.,* in acidosis, and in the integrative action of the blood, *e.g.,* in the control of breathing. In nephritis we found a condition where changes have perhaps reached a maximum both in magnitude and in extent.

We shall do well to note before proceeding that adaptive processes may be carried out in different ways, thus blood-flow and coefficient of utilization of oxygen may vary inversely, the lung may take over regulatory control that is ordinarily performed by the kidney, and it is well known that the conscious mind may likewise assume control of processes that are generally otherwise carried out. An instructive and amusing example of this type of rela-

tion in the field of economics is given by Pareto's proof[158] that "the social function of speculators, in so far as they do not act directly upon prices, is to solve as promptly and as well as possible the equation of economic equilibrium. It is these same equations that the employees of a socialistic state would have to solve." In sum, problems of adaptation are to be considered mathematically in relation to the state of equilibrium of the whole organism, and they present themselves alike in the biological and in the social sciences. This fact should suffice to rule out entelechies and vitalism.

* * * * *

In blood there is one instance of adaptation, to which I have in the past devoted much thought, that seems to take a place by itself. This is the fitness of the properties of water and carbon dioxide, in concentration and metabolic importance the chief components of the organism, for the constitution of stable physico-chemical systems. The most obvious facts are the solvent power of water and the buffer action of bicarbonates, both of which manifest themselves in all natural water and all organisms, and constitute two of the great factors of the geological and biological history of the earth.

These properties of the two substances depend upon the ensemble of properties of the elements hydrogen, carbon, and oxygen. The properties of these three elements "lead to the presence of water and carbon dioxide in the atmosphere, and to the meteorological cycle. This cycle regulates the temperature of the globe more perfectly than it could be regulated by any other substances concerned in any other similar cycle. It produces an almost constant temperature in the ocean, as well as constancy of composition and of alkalinity. It mobilizes all over the earth great quantities of all the elements; it deposits them in great variety and inexhaustible profusion in the

[158] P. Boven, *Les Applications Mathématiques à l'Économie Politique,* p, 154, Lausanne, 1912.

ocean; it comminutes and disperses all kinds of insoluble minerals, thereby diversifying the land; it causes water to penetrate and to remain in nearly all localities; and all of these processes are more perfect or more extensive than they could be if a large number of the different properties of water were not what they are. Thereby the greatest variety and quantity of structural materials are accumulated. Meanwhile the conditions which make for durability of structures are also assured.

"Other similar results depend upon the chemical properties of these three elements. Such properties lead to an even greater variety of chemical combinations and chemical reactions, to an unequalled diversity of properties in their products, and to qualitatively and quantitatively important transformations of energy.

"Out of all these substances, inorganic and organic alike, as a result of the properties of water and of carbon dioxide, the construction of an almost infinite diversity of phases and systems is possible. Natural phases and systems may both vary almost indefinitely in number and variety of components, in concentrations, and in configurations. They may be so constituted as to produce the most varied forms of activity. Like their components they may manifest the greatest diversity of properties. . . . These and many other things depend upon the properties of hydrogen, carbon, and oxygen. . . . Each of these properties is almost or quite unique, either because it has a maximum or a minimum value or nearly so, among all known substances, or because it involves a unique relationship, or an anomaly. No other element or group of elements possesses properties which on any account can be compared with these. . . . Thus we reach the conclusion that the properties of hydrogen, carbon, and oxygen make up a unique ensemble of properties each one of which is itself unique. This ensemble of properties is of the highest importance in the evolutionary process, for it is that which makes diversity possible. To this end it provides materials, and in large measure the necessary sta-

bility of conditions. . . . The unique ensemble of properties of water, carbonic acid, and the three elements constitutes, among the properties of matter, the fittest ensemble of characteristics for durable mechanism.'"[159] For these facts I have no explanation to offer. All that I can say is that they exist, that they are antecedent to organic adaptations, that they resemble them, and that they can hardly be due to chance.

The properties of water and of carbon dioxide are especially related to the theory of *milieu intérieur*. We shall now return to this theory, which is perhaps destined to play an even greater rôle in the future than it has in the past. The action of blood, regarded as environment of the tissues, may be considered as integrative. This is true alike for the first approximation which regards the physico-chemical properties of the system as constant, and for the closer approximation which takes into account normal gradients and the changes which accompany activity and disease. It has, however, not been customary to take into account the integrative activity of blood considered as a physico-chemical system. Such activity has hardly been attributed to this substance otherwise than in its action as a vehicle for the transfer of hormones which, together with the integrative action of the nervous system, includes the modes of integration that are usually studied. An exception may be noted in the participation of blood in the control of breathing, and other exceptions might be cited, but the general problem has not been considered.

As a means of beginning the investigation, a generalized description of the physico-chemical system may be attempted. There are two phases, plasma and cells. There are eight components. Four of these, water, carbon dioxide, hydrochloric acid, and oxygen, are present in both phases and, as the state of the equilibrium changes, in some form or other pass back and forth between the two

[159] L. J. Henderson, *The Order of Nature,* chap. X, Cambridge, Mass., 1917.

phases. Two components, plasma base and plasma protein, are present in plasma only; two others, cell base and cell protein, only in the cells. The first order relations between these components may be regarded as (1, 2, 3, 4) the diffusion of the four first-mentioned components across the cell walls, the acid-base reactions in plasma (5, 6, 7) and in cells (8, 9, 10) between base and carbon dioxide, hydrochloric acid and proteins, finally, (11) the reaction in the cells between oxygen and hemoglobin. All these relations are diagrammatically represented on figure 224. We shall disregard all other components and all second order relations, of which many exist, between the designated components.

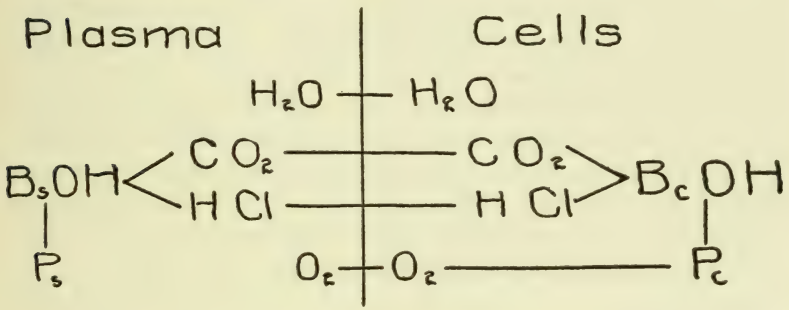

Fig. 224

Blood as a System

In order to conceive the conditions of equilibrium in this system we may adopt the following aid to the imagination: Each component in each phase is regarded as a sphere attached by an extensible elastic band at some point on a motionless rigid support; the bonds shown in the figure are conceived as other similar bands joining the several spheres, in accordance with the disposition of the bonds of the figure; the elastic bands are of different lengths and sizes, the points of attachment to the support variously disposed, in accordance with the properties of

the physico-chemical system. The resulting condition of equilibrium is readily imagined. Variation of equilibrium resulting from variation of the masses of the components may be represented by varying the mooring bands in length, thickness, and points of attachment to the rigid support.

Let us consider the transfer of oxygen by the system represented in figure 224 and, in order to fix our ideas, let us take affinity of hemoglobin for oxygen as an index of the magnitude of the process. It has been shown both theoretically and experimentally in these chapters that the value of k which measures this affinity is a function of the hydrogen ion concentration of the cells. It is true that k is also a function of other variables, but this fact may be provisionally neglected. Now hydrogen ion concentration of the cells has been shown both theoretically and experimentally to be a function of the concentration in the cells of the components, carbon dioxide, hydrochloric acid, oxygen, base, and protein. These facts are represented on figure 225, which is obtained from figure 224 by the addition of the representation of the hydrogen ion in the cells and of the relations just specified.

Evidently the hydrogen ion concentration of the cells, and therefore the value of k, may be varied by varying the

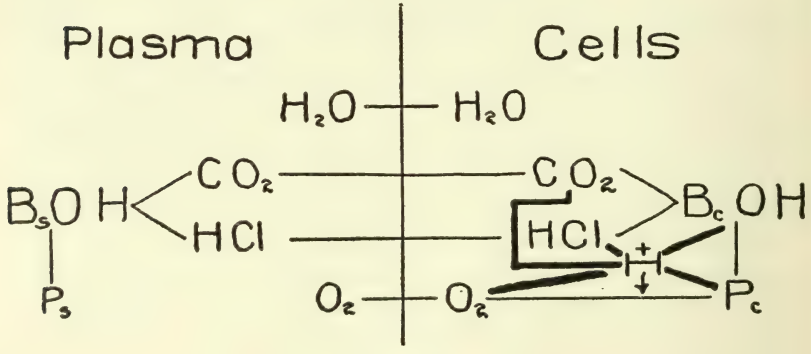

Fig. 225

Blood as a System: The Hydrogen Ion Relations

concentration of any of the components (except water for which the effect is small) in either cells or plasma. The effect is in some cases direct, in others indirect. Nothing in this discussion enables us to infer the magnitudes of the different effects. However, it is a fact that the indirect effect produced by varying the concentration of P_s tends to be small. Again, physiological conditions permit wide variation in oxygen and carbon dioxide pressures. Hence the effects produced by varying the concentrations of these substances may be large. For chemical reasons they are likely to be larger when carbon dioxide pressure is varied than when oxygen pressure is varied. Again, for physiological reasons, variation in hydrochloric acid concentration is likely to be smaller and the effects correspondingly so. Base is here taken as the base associated with the three specified acid components. When, as in fatigue and in disease, other acids enter the blood, or when pathological changes in the component hydrochloric acid take place, other effects upon the value of k may also be noted. With the help of a complete nomogram, or still better, of all the nomograms that we have studied, it would be possible to carry through at least a partial quantitative analysis of the question. But this is not the object of the present discussion. That object is but to illustrate in a convenient diagram the fact that no single factor of the plasma can be regarded as determining the affinity of hemoglobin for oxygen, or as being the cause of variations of this affinity.

This conclusion is an obvious corollary of all our previous conclusions, but the facts will repay a closer scrutiny. Let us note, to begin with, that even when a single component is varied, the effects upon the hydrogen ion concentration of the cells are both direct and indirect, and defy analysis except by means of a nomogram. Thus, for instance, variation in partial pressure of carbon dioxide has been shown to produce variation in carbon dioxide, hydrochloric acid, and oxygen concentrations within the cell. Therefore, the hydrogen ion concentration of the

cells is determined by the system as a whole, just as a scrap of paper is cut by a pair of shears and not by one of the blades. This conclusion could also have been deduced from the work of Gibbs. Indeed, if we adopt the physico-chemical standpoint it is a mere truism.

We may now recall the fact that the hydrogen ion concentration of the cells is not the only variable upon which the value of k depends, so that we cannot associate this quantity with a single variable even in the cells. Thus we have reached the result that the affinity of hemoglobin for oxygen in blood depends upon the composition of the whole system, that every variation in this affinity is accompanied by a variation of the condition of the whole system and that every variation in the system is accompanied by a variation of this affinity.

This result may now be applied to the problem of the control of breathing. Provisionally, let us adopt Gesell's hypothesis that the hydrogen ion concentration of the cells of the respiratory center determines the ventilation of the lungs. Then we may make use of figure 225 for this case also. The left-hand side of the figure will now stand for the composition of blood plasma and lymph, the right-hand side of the figure for the interior of the cells of the respiratory center. The diagram is now a less accurate approximation, for lymph has not the same composition as blood plasma and nerve tissue differs widely in composition from blood cells. But these facts are irrelevant to the present discussion for, as we saw in the first chapter, the components of blood to which we have limited our discussion are, except for differences in the proteins, the components of all protoplasm. However, the conditions of diffusion across the cell walls are also different. For this reason, we must regard the bonds of figure 225 as not all precisely determined. Finally, within the nerve cells oxygen does not combine with protein as it does in the erythrocytes; but it does nevertheless enter into the processes of metabolism in the cells. Therefore, it is somehow related, directly or indirectly, to each of the other compo-

nents. In short, the diagram represents the facts less concretely, but hardly less effectively, than before.

It is now obvious that on the present theory there can be no specific respiratory hormone or stimulus and that the blood as a whole is the stimulus of breathing. But, further, if within the red cells the union of oxygen with hemoglobin is a function of the state of the whole system, then, since the second process is probably more complex than the first, it is at least possible, even extremely probable, that the stimuli sent out by the cells of the respiratory center also depend upon the whole state of these cells, and not merely upon the concentration of the hydrogen ion or of any other single substance. I think we should go farther than this, generalize the conclusion and set it up as a postulate. As a working hypothesis it is capable of being very useful.

To this end figure 225 may be used not only as an illustration of the relations between blood and the respiratory center but also generally as an illustration of the relations between body fluids and tissues. Again it must be noted that the components vary slightly from case to case, that the conditions of diffusion also vary, and that consequently the direct relations between components are slightly variable.

Thus we have reached a very general conclusion. For it is now evident that, through the relations illustrated by the figure and the motion of the blood in the circulation, there is established a physico-chemical integration of the organism which seems to be more primitive than any other form of integration. The changes in composition of blood during work which we have studied in previous chapters suggest how rapid and how extensive the processes thus involved may be.

In its simplest form such integrative action may be conceived as follows: The blood exists in a state of heterogeneous equilibrium with all the cells of the body. Suppose a change to occur in some part, say increase of activity in a group of muscles. Then the physico-chemical

equilibrium within these muscles will change, and this must be accompanied by changes in that portion of the blood which is in heterogeneous equilibrium with the varied muscle cells. This blood is carried back to the heart and mixes with all the other venous blood. Changes in arterial blood result and these lead to changes not only in the respiratory center, but in all other parts of the body. For example, venous blood, coming from inactive regions of the body during a period when muscles in other regions are active, may contain less lactic acid than arterial blood. Other changes in the acid-base equilibrium of the system are also involved, for here the inactive portions of the body are serving as buffers. Such considerations are far too simple and obvious to have escaped notice, but they have not been clearly conceived or methodically studied. Also it has not been noted that every part of the body has at its disposal the resources of the whole body, or at least it has not been clearly perceived in what manner this is the case when disturbances of the more general features of the physico-chemical system are in question. In accordance with the principle quoted from Pareto, which can also be stated, though less completely, in the form of the theorem of Le Chatelier, such processes are regulatory or compensatory, and tend not only to check but also to reverse the variations, and to restore the original state. If we would understand the mechanism of these integrative processes it is necessary to take account of at least the more important properties of the physico-chemical system. For this purpose figure 224, or better a complete nomogram, should be employed. When the phenomena have been extensively studied in this manner, as we have studied them for blood alone in these chapters, the synthesis of an enormous mass of existing physiological knowledge will perhaps become possible.

* * * * *

In the beginning we were confronted with Claude Bernard's question: What is "the elementary condition of

the phenomena of life''? At the end of this long study an answer may be given. This answer was first formulated provisionally as an aid in our work; as we proceeded we found it useful and, though always incomplete, never erroneous. We can now see that, though it is surely destined to be replaced by a better answer to the question, it will still serve for a brief time to come.

The elementary condition of the phenomena of life is a particular kind of physico-chemical system. A description of this kind of system, so general as to be almost but not quite useless, is given in figure 224. A more complete description of an unrepresentative member of the class is given by a blood nomogram such as figure 41. Although the greater part of this book has been devoted to the elucidation of the properties of this class of systems, a comprehensive and detailed description is as yet unattained.

There are many grounds for objecting to this statement. Some will still say that the elementary condition of the phenomena of life is the cell. Others will prefer a metaphysical definition. To the latter objectors it may be replied that the physiologist is seeking his own ends; to the former that this is but a question of means to those ends, and that for the present rational, mathematical, and physico-chemical studies of equilibria and stationary states in living organisms are fruitful of the results that he seeks.

APPENDIX

BY

DAVID BRUCE DILL

APPENDIX

IN studying the physico-chemical properties of blood it is frequently desirable to determine for a single specimen a complete set of carbon dioxide dissociation curves and two or three oxygen dissociation curves. This may be particularly true in the case of a pathological subject whose blood changes from day to day. We propose to describe the technique that we have used when as much as 135 cc. of blood and four investigators were available. There will also be described an experiment in which fairly complete information was obtained by one investigator on 30 cc. of blood.

Tonometers in which the blood is to be equilibrated are ordinarily prepared a day in advance. These tonometers are of the Barcroft type, of 250 to 350 cc. capacity and are illustrated in figure 1. The capacity is etched on each and serves as an identifying mark. The dry tonometers, with stop-cocks well lubricated and held in by rubber

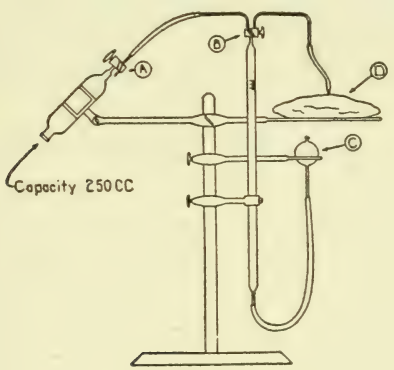

Fig. 1

Apparatus for Preparation of Gas Mixture

bands, are fitted with rubber stoppers. They are divided into three groups: I, for oxygenated carbon dioxide curves; II, for reduced carbon dioxide curves; and III, for oxygen curves. Tonometers in the last two groups are flushed out with nitrogen before stoppers are inserted.

In table 1 there are indicated the desired pressures of carbon dioxide and oxygen, the volume of each gas to be added and the volume of blood to be used.

A few comments may be made on table 1. No carbon dioxide need be added to tonometer *a* since enough will be freed from 18 cc. of blood to produce a carbon dioxide pressure of from 10 to 20 mm. Similarly, slight corrections in volume of carbon dioxide to be introduced into tonometers *i* to *l* are necessary. Ordinarily there is no occasion for adjusting the oxygen pressure carefully in group III tonometers. The oxygen pressures in the low range will exceed those calculated, in part because some oxygen is given up by the blood and in part because the nitrogen used in flushing out the tonometers is not oxygen free.

An additional tonometer, filled with nitrogen alone, is required for preliminary reduction of the blood to be used in tonometers *f*, *g*, and *h*.

The apparatus for preparing the gas mixture in a tonometer is shown in figure 1. The process may be illustrated with tonometer *i*. Let $i = 250$ and $B = 750$. Then $\frac{20i}{B} - 0.8 = 5.9$ and $\frac{10i}{B} = 3.3$. With tonometer stop-cock *A* open to the air and with stop-cock *B* of the gas pipette (50 cc. capacity, calibrated in 0.1 cc.) open to *A*, the gas pipette is filled with mercury with leveling bulb *C*. Stop-cock *A* is turned and 9.2 cc. (5.9 + 3.3) of gas drawn from the tonometer into the pipette. Stop-cock *A* is turned, and through its side outlet, this gas is discharged. Stop-cock *B* is turned to rubber bag *D*, containing oxygen. The pipette and connecting tube *AB* are flushed out with

TABLE 1.

Preparation of tonometers for equilibration of blood.

Purpose	Blood Used	Tonometer Number and Volume	pCO_2 *mm.*	pO_2 *mm.*	Vol. CO_2 to add *cc.*	Vol. O_2 to add *cc.*	Vol. Blood *cc.*
Oxygenated CO_2 curve.	Venous	*a*	10	air	none	none	18
		b	40	air	$\frac{40\,b}{B^*}$	none	4
		c	100	air	$\frac{100\,c}{B}$	none	18
Oxygenated CO_2 curve.	Arterial	*d*	40	air	$\frac{40\,d}{B}$	none	4
		e	70	air	$\frac{70\,e}{B}$	none	4
Reduced CO_2 curve.	Venous	*f*	10	0	$\frac{10\,f}{B}$	none	18
		g	40	0	$\frac{40\,g}{B}$	none	4
		h	100	0	$\frac{100\,h}{B}$	none	18
O_2 curve at pCO_2 = 20 mm.	Venous	*i*	20	10	$\frac{20\,i}{B} - 0.8$	$\frac{10\,i}{B}$	4
		j	20	30	$\frac{20\,j}{B} - 0.9$	$\frac{30\,j}{B}$	4
		k	20	40	$\frac{20\,k}{B} - 1.0$	$\frac{40\,k}{B}$	4
		l	20	60	$\frac{20\,l}{B} - 1.1$	$\frac{60\,l}{B}$	4
O_2 curve at pCO_2 = 40 mm.	Venous	*m*	40	15	$\frac{40\,m}{B}$	$\frac{15\,m}{B}$	4
		n	40	30	$\frac{40\,n}{B}$	$\frac{30\,n}{B}$	4
		o	40	40	$\frac{40\,o}{B}$	$\frac{40\,o}{B}$	4
		p	40	60	$\frac{40\,p}{B}$	$\frac{60\,p}{B}$	4
O_2 capacity	Venous	*q*		air	none	none	4
O_2 capacity	Arterial	*r*		air	none	none	4

* Barometer reading.

oxygen twice through the side arm of *A* and finally exactly 3.3 cc. of oxygen is measured into the pipette and then delivered into the tonometer. The bag of oxygen is replaced with a bag of carbon dioxide and after flushing out the pipette and connections through the side outlet of *A*, 5.9 cc. of CO_2 is introduced into the tonometer. The final pressure in the tonometer will vary but little from atmospheric pressure.

When more than 10 or 15 cc. of gas is withdrawn from the tonometer, account should be taken of the lowered pressure. This may be done approximately by suitable lowering of the leveling bulb or the volume of gas withdrawn may be measured with *B* closed. Such a set of tonometers as shown in table 1 may be prepared in about one hour.

We use 50 cc. centrifuge tubes for collection of the blood. Ten cubic centimeters of paraffin oil and 10 mg. of heparin* are added. This will take care of 30 to 40 cc. of blood with a suitable factor of safety. For certain purposes correction may be necessary for substances present in heparin, as the following analytical determinations indicate:

Total base, *millimoles per 30 mg. heparin,*	0.1
Total nitrogen, *mg. per 30 mg. heparin,*	2.3
Non-protein nitrogen, *mg. per 30 mg. heparin,*	2.3
Chloride, *millimoles per 30 mg. heparin,*	0.03
Phosphorus, *mg. per 30 mg. heparin,*	0.0
Calcium, *mg. per 30 mg. heparin,*	0.2

Assuming heparin has been added in the proportion of 30 mg. per 100 cc. of blood, these corrections may be simply applied.

After establishing local anesthesia with one per cent novocaine, blood is withdrawn, usually in two portions. One portion of about 20 cc. is taken from the radial or

* Heparin may be obtained from Hynson, Westcott, and Dunning, Baltimore.

brachial artery and a second portion of 115 cc. from an arm vein.

Particular care is taken in the case of the arterial blood to avoid contact with air. The syringe is turned up, the needle replaced with a glass tube 2 × 100 mm. and blood pushed through to the tip of this tube. The syringe is inverted and with the tube leading into the oil in the 50 cc. centrifuge tube, the blood is rapidly transferred. It is then stirred carefully until the heparin is dissolved. Venous blood is handled in the same manner, but less importance is attached to preventing access to air. The point is that any specimen of arterial blood normally represents the blood leaving the lungs while in respect to its oxygen and carbon dioxide contents arm vein blood may differ greatly from *vena cava* blood. Consequently it is of great interest to know the carbon dioxide and oxygen content of arterial blood and of but little interest to secure such information with regard to arm vein blood.

At this point one man proceeds promptly to the determination of the carbon dioxide and oxygen contents of the arterial blood. At the same time two others begin equilibrating blood. So far as possible, the blood awaiting equilibration is kept at a temperature of about 5°C.

With a 5 cc. syringe, the indicated amount of arterial blood is introduced into tonometer *d* through its stop-cock. With another syringe, mercury is forced through the stop-cock until the capillary bores are exactly filled. The time is noted and the tonometer is placed in the constant temperature bath where it is held in the mechanical rotator,* illustrated in figure 2. Tonometer *e* is handled in a similar manner.

Forty cubic centimeters of venous blood is then added to the nitrogen-filled tonometer and after sealing with mercury this is placed in the bath. Each of tonometers *a*, *b*, and *c* is then prepared and its equilibration begun. Twenty minutes' equilibration at 37.5° insures practically

* Manufactured by Warren E. Collins, Inc., Boston.

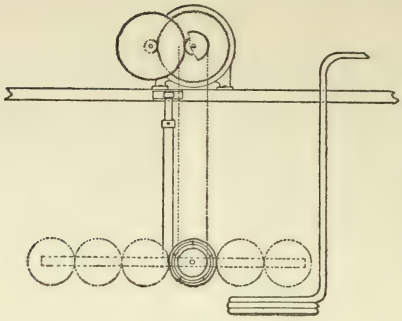

Fig. 2
Mechanical Rotator

complete equilibrium under the above-defined conditions. Accordingly, when tonometer *d* has been equilibrated for 20 minutes, it is clamped in an upright position in the bath and a blood sampling tube is attached with a short piece of small-bore rubber tubing. This sampling tube is like the gas sampling tube shown in figure 3B except that its capacity is only 6 cc. The tonometer stop-cock is turned to the side arm, the sampling tube stop-cock is opened and by raising the leveling bulb some mercury is forced through the connection. The tonometer stop-cock is then turned and, by lowering the leveling bulb, the blood is drawn into the sampling tube. After closing both stop-cocks, the rubber connection is removed and sampling tube and tonometer are taken from the bath. The sampling tube is placed in a properly denoted position in a rack and sealed off with mercury. The blood samples are held at about 5° until analyzed. The gas in the tonometer is analyzed as described below.

The tonometer used for preliminary reduction of 40 cc. of blood is taken from the bath after 20 minutes, dried and clamped in an upright position. The blood from it is used for filling tonometers *f*, *g*, and *h*.

So in turn all the blood is equilibrated. The technique for withdrawing blood from tonometers is modified in the

case of *a, c, f,* and *h.* A dry 15 cc. centrifuge tube graduated in 0.1 cc. is filled with paraffin oil and placed in a clamp near the bath. Of the total of 18 cc. of blood in the tonometer, approximately 4 cc. is drawn into the sampling tube. This and the rubber connection are removed. The tonometer is lifted from the bath and immediately covered with several thicknesses of a warm dry cloth. The stop-cock is exposed and dried, one or two drops of blood allowed to escape and the remainder transferred carefully into the centrifuge tube with the tip of the stop-cock under oil. Care must be exercised to close the stop-cock of the tonometer before the last bit of blood has escaped. Most of the excess oil is then displaced by inserting a one-hole rubber stopper. A glass rod with a flattened tip is pushed into the hole. Centrifugation is promptly begun and carried on for one hour at a speed of 2500 to 3000 r.p.m. After centrifugation the tubes are removed, blood volume and cell volume are observed and determination of carbon dioxide content of plasma is begun immediately.

When equilibration of blood is completed, the two men who have been so engaged begin blood gas analysis. Blood in a sampling tube is thoroughly mixed. The stop-cock is carefully opened until all mercury is expelled from the capillary. A No. 0 rubber stopper with small bore makes a satisfactory connection with the Ostwald pipette. (This pipette is of one cubic centimeter capacity and is calibrated between marks.) The leveling bulb is supported in the rack slightly above the level of the sampling tube stop-cock so that there is sufficient pressure to fill the pipette when the stop-cock is opened. After the blood sample has been introduced into the Van Slyke apparatus the sampling tube is again sealed off with mercury. For the actual operation of determining carbon dioxide and oxygen we follow the methods of Van Slyke and his associates without deviation (1, 2, 3).

Such an experiment involves 26 single carbon dioxide determinations and 22 oxygen determinations. Under the

conditions described these determinations can be completed within seven hours of the time equilibration is begun.

A fourth investigator analyzes the gas phase of the tonometer. The tonometer, cooled to room temperature, is connected to the Haldane apparatus. The stop-cock is carefully opened and, without mixing, 7 cc. of gas is drawn into the pipette. The pressure, as measured by the difference in mercury levels, is read with a mm. rule. The gas in the pipette is then discharged through the side arm of the tonometer stop-cock, two additional small portions of gas are used for flushing out and then a sample is drawn into the Haldane apparatus and analyzed in the usual manner for carbon dioxide and oxygen. A check analysis is also made. During the analysis the room temperature is noted. The pressure of carbon dioxide in the tonometer during equilibration is calculated as follows:

$$pCO_2 = \%CO_2\,(B - pH_2O + \Delta p) \times \frac{273 + t_e}{273 + t_r} \times \frac{(v + 6)}{(v - b)}$$

where B = barometric pressure in mm. Hg.

pH_2O = water vapor pressure at room temperature in mm. Hg.

Δp = tonometer pressure as measured in the Haldane apparatus.

t_e = temperature of equilibration, °C.

t_r = temperature of room, °C.

v = tonometer volume in cc.

b = volume of blood equilibrated in cc.

The term $v + 6$ is explained as follows: The volume of the tonometer is virtually increased by 7 cc. when Δp is measured and is less than its dry volume by 1 cc. because approximately that volume of blood adheres to its walls. The above formula is essentially that of Bock, Field, and Adair (4). In a similar manner pO_2 is calculated.

As soon as carbon dioxide content of each plasma has been determined in duplicate, a 2 cc. portion of each is

pipetted into an Erlenmeyer flask of capacity 125 cc. for determination of total chloride. The method of Van Slyke (5) is employed. The remaining plasma is pipetted off and combined. It may be set aside at 5°C. and, at leisure, determination may be made of water by drying at 110°, of total base by the method of Fiske (6) as adapted to serum, total nitrogen by Kjeldahl, and non-protein nitrogen by Van Slyke (7). In some cases we have determined sodium plus potassium by Stoddard's method (8) and potassium by Fiske's method (private communication).

After the plasma has been removed from the cells, the supernatant paraffin oil and traces of plasma are removed as completely as possible with a pipette and finally with rolled filter paper. About 2 cc. of each specimen of cells is then pipetted into a weighed 125 cc. Erlenmeyer flask. The flasks are weighed and chloride determination is carried out by Van Slyke's method.

In order to calculate cell chlorides to the volume basis, specific gravity of the cells must be determined or calculated. The equation

$$G_c = 1.3 - 0.3(H_2O)_c$$

is sufficiently accurate for most purposes. In this equation G_c = specific gravity of the cells. $(H_2O)_c$ = water of the cells expressed as gm. H_2O/gm. cells, as determined by drying at 110°.

An additional sample of cells is used for determination of total base by an application of the method of Fiske (5).

In some such manner as this the experiment on T.J.F. discussed in chapter VI was carried out. While the plan of that experiment differed in certain particulars from the plan just described, the points of departure are not important.

We may turn now to an experiment carried through by one investigator in the course of six or seven hours with 30 cc. of blood. The details are shown in table 2.

TABLE 2.

The fewest data required for an approximately complete physico-chemical description of blood.

A typical experiment on 30 cc. blood.

A. CARBON DIOXIDE DISSOCIATION CURVES.

Tonometer	Vol. of blood used *cc.*	pCO_2 *mm. Hg*	pO_2 *mm. Hg*	Total CO_2 Whole blood *vol. %*	Total CO_2 True plasma *vol. %*	Cell volume *cc./l. blood*
a	15	45.1	air	46.5 46.3	55.4	522.0
b	3	74.6	air	56.75		
c	3	42.6	5.0	50.4 50.7		

B. OXYGEN DISSOCIATION CURVE.

	Vol. of blood used	pCO_2	pO_2	Total O_2	HbO_2	$\%HbO_2$
d	3	41.1	24.8	7.80	7.72	32.9
e	3	40.9	46.1	18.55	18.43	78.6
f	3	0.0	air at 20°	23.95	23.45	100.0

C. OBSERVATIONS ON PLASMA AND CELLS OF TONOMETER *a*.

Plasma H_2O, *gm. per liter plasma,* 926
Plasma chloride, *millimoles per liter plasma,* 96.4
Cell H_2O, *gm. per liter cells,* 708
Cell chloride, *millimoles per liter cells,* 47.0
Plasma total base, *millimoles per liter plasma,* 165
Plasma protein, *mg. per liter plasma,* 93.7

D. ALVEOLAR $pCO_2 = 38$ mm. Hg.

With this fairly comprehensive experiment and with knowledge regarding the "law of the blood" one can synthesize fairly accurately a nomographic description of this blood.

There remains to be described our method for determining rate of blood flow. This determination in man fixes the position on the d'Ocagne nomogram of the venous line. With animals one needs for this purpose only to

draw mixed venous blood from the heart. In either case the arterial line may be determined by direct examination of arterial blood.

First of all one determines the rate of carbon dioxide production. After the subject has reached a steady state, all air expired in a given time is collected. Its content of CO_2 is estimated by measuring its volume and determining percentage composition of a representative sample, using the Haldane apparatus.

Rate of blood flow can be calculated when one knows, in addition to the rate of carbon dioxide production, the carbon dioxide content of arterial and of venous blood.

The carbon dioxide content of arterial blood has been determined directly on many occasions. It is now known that it can be determined quite accurately by an indirect method. Thus, in resting subjects, Bock and Field (9) showed that alveolar air collected by the Haldane-Priestley method at the end of normal expiration had a carbon dioxide pressure averaging 0.5 mm. less than arterial blood. Similarly, Dill, Lawrence, Hurxthal and Bock (10) found that in work ranging up to ten times the resting level Haldane-Priestley alveolar air collected at the beginning of expiration had practically the same pressure as arterial blood. Thus the carbon dioxide pressure of arterial blood can be established indirectly and the carbon dioxide content estimated by reference to the carbon dioxide dissociation curves.

Carbon dioxide content of venous blood is determined indirectly by a method which awaits direct confirmation. To five or six liters of oxygen enough carbon dioxide is added to approximate its pressure in venous blood. This mixture is rebreathed by the subject for a preliminary period slightly shorter than the time required for an average complete recirculation. Figures 3A and 3B represent the apparatus used. In work experiments the small rubber flap valves are replaced by similar valves of a larger size.

When the subject has reached a steady state, with

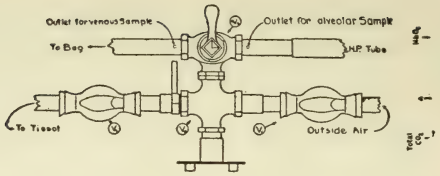

Fig. 3A

Disposition of Valves

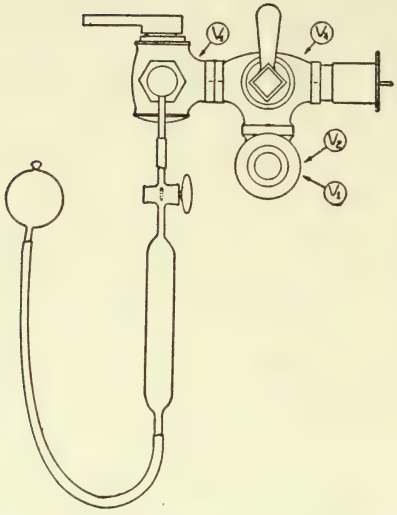

Fig. 3B

Sampling Device

valve V_4 open to tube HP, V_3 is opened to V_4 and simultaneously the signal, "Blow!" is given. In rest the signal comes just as the normal expiration is completed; in exercise, just as it begins. The normal expiration is thus slightly prolonged into a forced expiration in rest while in work the forced expiration is completed at the time a normal expiration would have been completed. Precisely at the end of this forced expiration, V_4 is opened to the bag and the signal "In!" is given. The subject makes a

rapid maximum inspiration and holds it until the signal, "Out!" is given when he makes a rapid maximum expiration into the bag. V_3 is then turned and normal respiration reëstablished.

The gas sample representing arterial blood is drawn into one sampling tube and that representing oxygenated venous blood is drawn into the other by displacement of mercury in each case. A series of four to six sets of such samples are usually collected.

The assumption is made that it is possible to oxygenate venous blood and establish an equilibrium between carbon dioxide of air and of blood before appreciable recirculation of blood. We have indirect evidence justifying such an assumption and indicating that the equilibrium time approximates 15 seconds in normal resting man and decreases in a linear fashion to a value ranging from five to seven seconds when the metabolic rate is ten times its resting value. The evidence is in the course of publication by Bock and his associates.

BIBLIOGRAPHY

1. Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, LXI, 523.
2. Van Slyke, D. D., *J. Biol. Chem.*, 1927, LXXIII, 121.
3. Van Slyke, D. D., and Sendroy, Julius, Jr., *J. Biol. Chem.*, 1927, LXXIII, 127.
4. Bock, A. V., Field, H., Jr., and Adair, G. S., *J. Biol. Chem.*, 1924, LIX, 353.
5. Van Slyke, D. D., *J. Biol. Chem.*, 1923, LVIII, 523.
6. Fiske, C. H., *J. Biol. Chem.*, 1922, LI, 55.
7. Van Slyke, D. D., *J. Biol. Chem.*, 1927, LXXI, 235.
8. Stoddard, James L., *J. Biol. Chem.*, 1927, LXXIV, 677.
9. Bock, A. V., and Field, H., Jr., *J. Biol. Chem.*, 1924, LXII, 269.
10. Dill, D. B., Lawrence, J. S., Hurxthal, L. M., and Bock, A. V., *J. Biol. Chem.*, 1927, LXXIV, 313.
11. Henderson, Y., and Haggard, H. W., *Am. J. Physiol.*, 1925, LXXIII, 193.

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